
St. Marys River and Lime Island

St. Marys River Area of Concern (Canadian section)

Beneficial Use Impairment Redesignation:
Bird and Animal Deformities or Reproductive Problems

December 2014

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ST. MARYS RIVER

EXECUTIVE SUMMARY

This document serves as the official record to redesignate, on the Canadian side of the St. Marys River Area of Concern (AOC), the *Bird and Animal Deformities or Reproductive Problems* beneficial use impairment to NOT IMPAIRED. This is a positive change from the previous designation of REQUIRES FURTHER ASSESSMENT, which had been in place since the Stage 2 Remedial Action Plan report (December 2002).

The redesignation to NOT IMPAIRED on the Canadian side follows the same change by the United States Environmental Protection Agency in February 2014, stemming from a separate assessment of the beneficial use completed by the Michigan Department of Environmental Quality in 2012.

Based on four years of study by Environment Canada's Wildlife Toxicology unit, there is no evidence of impairment in colonial waterbirds attributable to local contamination effects within the AOC, and the reproductive success for birds studied within the AOC is similar to that from outside the AOC.

The attached technical report with addendum details the study methods, field and laboratory analysis, results, and discussion and conclusions from Environment Canada's multi-year assessment. Its evaluation of deformities, contaminant levels and reproductive health of birds on the Canadian side of the river provides the rationale for changing the designation. Key findings include:

- No physical deformities have been detected in herring gull or common tern chicks or adults.
- There is a low incidence of embryonic deformities that cannot be linked to contaminant burdens or to geographical area (i.e., there is no significant difference between AOC and non-AOC bird colonies).
- Contaminant levels are low overall, and not sufficiently elevated to have an adverse impact on reproductive success and development. This is the case for Polychlorinated biphenyls (PCBs) and other organochlorines, dioxins/furans, heavy metals like mercury, and Polybrominated diphenyl ethers (PBDEs).
- The reproductive success for herring gulls within the AOC is high, and that of the common tern is similar to the rest of the region.

The federal, provincial and state agencies that developed the Stage 2 Remedial Action Plan, with input and advice from the Binational Public Advisory Council, designated the beneficial use status as "Requires Further Assessment". This was due to Michigan State University researchers finding three cross-bill common tern chicks (n= 120) while sampling on Lime Island in 1998 (page 17). The Stage 2 report stated that while no other deformities had been noted in wildlife along the St. Marys River, the Lime Island findings warranted further assessment via the following monitoring actions (page 77):

- Action FFM-5: Complete an assessment of common and black tern populations for the area.
- Action FFM-6: Analyze contaminant levels in eggs from herring gull, black tern, and common tern nests in the AOC.
- Action FFM-8: Complete reproductive assessments of herring gulls, black terns and common terns, and an assessment of deformities in common terns within in the St. Marys River.

The attached technical report with addendum not only provides the rationale for changing the designation to NOT IMPAIRED, it also completes Actions FFM-6 and FFM-8. It must be acknowledged that although these two actions call for a study of contaminant levels and reproductive health within black terns, such an act requires removal of eggs from the colony. This is not an option for black terns, as it has been suggested they are experiencing declining numbers across the Great Lakes basin.

A separate report by Environment Canada has been completed that delivers on Action FFM-5. It concludes common tern and black tern populations are not unduly impacted within the St. Marys River compared to the North Channel of Lake Huron, or even the rest of Ontario and Great Lakes basin. The population assessment corroborates there are no reproductive problems for wildlife within the Canadian side of the Area of Concern, and as such, has been included in this redesignation report as further justification for changing the official status to NOT IMPAIRED.

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Assessment of the Wildlife Reproduction and Deformities Beneficial Use Impairment in the St. Marys River Area of Concern (Ontario)

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ABSTRACT

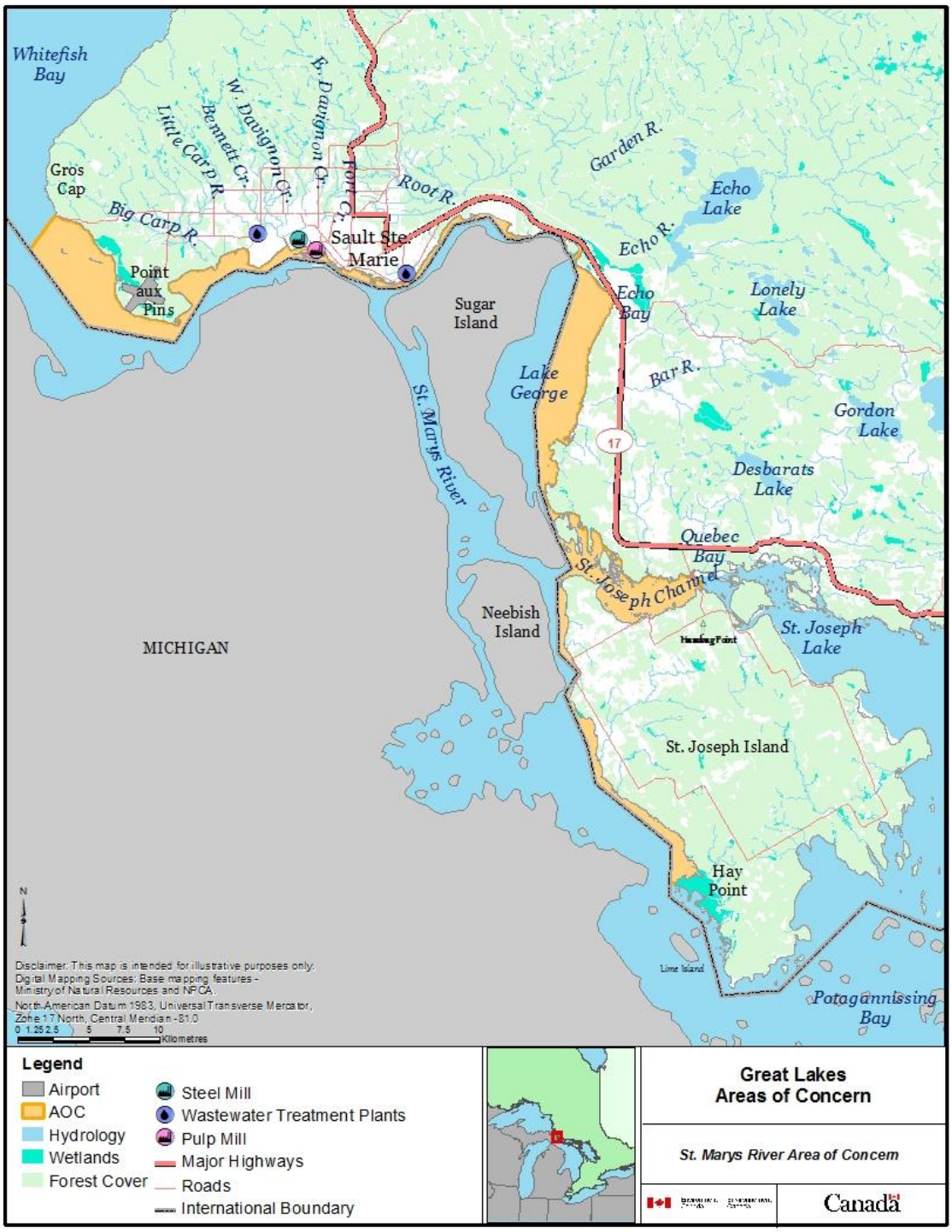
Reproduction and development were examined in herring gulls (*Larus argentatus*) and common terns (*Sterna hirundo*) breeding within the St. Marys River Area of Concern (Ontario) in 2011 and 2012. Freshly-laid eggs were collected from colonies within the Area of Concern (AOC) as well as outside of the AOC, artificially incubated in the laboratory and assessed for embryonic viability, incidence of embryonic deformities, contaminant burdens and other biochemical endpoints. Productivity was determined at the colonies when chicks were $\geq$ 21 days old and chicks were examined for morphological deformities as well as other biological endpoints. Overall, embryonic viability of herring gulls and common terns was high at AOC colonies and herring gull productivity at AOC colonies was within the range required to maintain a stable population. Common tern productivity at AOC colonies, while low, was consistent with rates for common terns within the region and was largely attributable to external stressors, such as predation and severe weather events. No morphological deformities were found in field surveys of juveniles of either species (based on sample sizes of 13-63 chicks). Frequencies of embryonic deformities were comparable between AOC colonies and reference colonies for both species. Comparable burdens of non-*ortho* PCBs, 2,3,7,8-TCDD, and TEQs were also found between deformed and normal embryos from AOC colonies of both species and suggest that embryonic deformities are not associated with exposure to dioxin-like PCBs and dioxins. Importantly, contaminant burdens (e.g., PCBs, 2,3,7,8-TCDD, and mercury) in gull and tern embryos from the St. Marys River AOC (Ontario) were comparable and not notably elevated compared to burdens at respective reference colonies in the two study years. Finally, concentrations of PCBs, other organochlorines, PBDEs, dioxins/furans and mercury were not sufficiently elevated in embryos to adversely impact the reproductive success and development of herring gulls and common terns foraging in the St. Marys River AOC.

INTRODUCTION

The St. Marys River is approximately 112 kilometres in length and is an important and major waterway in the Great Lakes interconnecting Lake Superior and the North Channel of Lake Huron. The St. Marys River Area of Concern (AOC) is one of 43 Great Lakes AOCs which were initially identified by Canada, the United States and the International Joint Commission (IJC) as specific locations where local environmental degradation had severely impacted the area's ability to support aquatic life. Historical discharges of pollutants from local steel and pulp and paper industries, a tannery and manufactured gas plant, and municipal storm sewers and wastewater treatment plants impaired water quality and contaminated sediment along parts of the St. Marys River (OMOE and MDNR 1992). Contaminants of concern included PAHs, mercury and other heavy metals, and polychlorinated biphenyls (PCBs) which contributed to exceedences of water quality objectives, sediment quality guidelines, fish consumption guidelines and impacted biota (OMOE and MDNR 1992; EC *et al.* 2002). The St. Marys River, as a connecting channel, is one of five Great Lakes AOCs jointly shared by Canada and the United States. As directed by Annex 2 of the 1987 Protocol to the Canada-U.S. Great Lakes Water Quality Agreement (GLWQA), a Remedial Action Plan (RAP) for the St. Marys River was developed collaboratively by Canadian and U.S. partners to address environmental concerns that are specific to the Ontario and Michigan portions of the river, respectively. Implementation of the remedial actions continues. The border of the Canadian portion of the St. Marys River AOC (Ontario) extends from its head at Gros Cap

in Whitefish Bay downstream to St. Joseph Island via Lake George to Quebec Bay in the St. Joseph Channel and downstream to Hay Point on the western shore of St. Joseph Island (Figure 1).

Figure 1. The St. Marys River Area of Concern as defined by the boundary in Ontario.



Fourteen beneficial use impairments (BUIs), caused by a detrimental change in the chemical, physical or biological integrity of the Great Lakes system, were used by the Canadian and U.S. federal governments to identify AOCs and then as a framework for directing remediation efforts. One of these BUIs, "bird or animal deformities or reproduction problems", relates to contaminant exposure or other anthropogenic environmental stressors on reproductive success or deformity rates in wildlife. Based on reports from the 1980s, there were no bird or animal deformities or reproductive problems in the St. Marys River AOC (with limited contaminants data available for wildlife) and this BUI was not impaired (OMOE and MDNR 1992). However, the status of the BUI in the AOC was subsequently changed to "requires further assessment" in the 1990s/early 2000s with the recommendation that reproductive assessments of herring gulls (*Larus argentatus*) and common terns (*Sterna hirundo*) be completed within the AOC (EC *et al.* 2002). It was recommended that deformities be assessed in common terns following evidence of deformities found in three common tern chicks - cross bills - at a colony on Lime Island, Michigan, in 1998. Following this, studies of the potential effects of contaminants on reproduction and development in aquatic-feeding wildlife were initiated by federal, state and provincial agencies to more fully evaluate and assess the current status of this BUI in the St. Marys River AOC.

Fish-eating wildlife, such as colonial waterbirds, are important indicators of exposure to persistent contaminants in the aquatic environment (Fox and Weseloh 1987). As top predators, they occupy a high trophic level in the aquatic food web and therefore can accumulate high levels of contaminants which may in turn adversely affect their reproductive health and development. Two colonial waterbird species which breed and forage within the St. Marys River AOC were selected for assessment purposes. The herring gull is a long-lived, primarily fish-eating colonial waterbird that from the time it reaches breeding age is a year-round resident in the Great Lakes basin. The common tern, an obligate piscivore, is an excellent bio-indicator for tracking potential problems relating to PCB contamination since they are very sensitive to the dioxin-like effects of certain PCBs (Nisbet 2002). In 2011 and 2012, breeding colonies of these two species were studied by Environment Canada (EC) in the St. Marys River AOC (Ontario). Eggs were collected for artificial incubation in the laboratory to assess embryonic viability, incidence of embryonic deformities, contaminant burdens and biochemical endpoints (i.e., fatty acids and stable isotopes). Under controlled laboratory conditions, this method assesses the effects of embryonic exposure to potentially high levels of contaminants during critical periods of development. Reproduction and development were also assessed in wild populations with visits to colonies to monitor productivity and examine chicks for morphological deformities as well as measure additional endpoints (i.e., stress hormone, thyroxine levels) relating to growth and development which could be influenced by increased contaminant exposure. In combination with the results of extensive reproduction and development studies conducted in Michigan's AOCs (MDEQ 2012), the results of this study will be used to assess the current status of the wildlife reproduction and deformities BUI in this binational AOC.

METHODS

Two herring gull colonies within the St. Marys River AOC (Ontario), Hay Point (46°07'N, 83°59'W) and Pumpkin Point (46°23'N, 84°07'W), and one downstream reference colony at Double Island (46°10'N, 82°52'W) in the North Channel of Lake Huron were selected for study purposes. The common tern colony at Hay Point was the AOC colony used in this study. A tern colony on North Sister Rock (46°18'N,

83°54'W) was selected as an alternate AOC colony following abandonment of the Hay Point colony (likely due to predation) early in the breeding season of 2011. This colony is in St. Joseph Lake and is approximately 6 km beyond the AOC boundary at Quebec Bay. A tern colony on Cousins Island (46°04'N, 82°49'W) in the North Channel was selected as the reference colony.

Visits to each colony were made at two times in the breeding season: 1) egg laying (late April for gulls and late May for terns) and 2) when chicks were $\geq$ 21 days old (mid-May for gulls and mid-June for terns) in 2011 and 2012 to assess reproduction and various parameters of health. In 2011, an additional visit in May (i.e., immediately post-hatch) was conducted to assess deformity rates but this was not performed in 2012 in order to minimize disturbance associated with visits to the colony. During the first visit, 15-30 freshly-laid eggs (i.e., not incubated) were collected from one-egg nests at each colony for artificial incubation in the laboratory at the National Wildlife Research Centre (NWRC) in Ottawa. Embryonic viability, incidence of embryonic deformities, contaminant burdens, fatty acid profiles and stable isotope signatures were determined. In addition, a thorough nest count was conducted and contents were recorded. Individual nest enclosures (~1m in diameter and 16" high) were constructed around ten to twelve 3-egg nests at each colony. As a measure of colony health, egg measurements for up to thirty 3-egg clutches were recorded (in millimetres) and egg volume calculated as:

$$\text{Egg volume } (cm^3) = K_{sp} \times (\text{length} \times \text{breadth}^2)/1000$$

where K_{sp}=0.476 and 0.502 for herring gulls and common terns, respectively.

Total clutch volume was determined as the sum volume of the three eggs in the clutch. Intraclutch variation in egg size was calculated as the difference in volume between the largest and smallest egg in the clutch divided by the largest egg size (i.e., volume) and multiplied by 100. During the second visit, when chicks in enclosed nests were $\geq$ 21 days old, productivity was calculated as:

$$\text{Productivity} = \text{no. of} \geq \text{21-day-old chicks/no. of enclosed nests}$$

Enclosed nests that had been abandoned or where there was evidence that chicks had escaped were not included in estimates of productivity. Body measurements of 15 chicks, including mass, tarsus, wing cord, and culmen length (only measured in 2012), were also recorded and chicks were banded with a stainless steel USFW band. A 2 ml blood sample was collected from the brachial vein of chicks using a heparinized syringe to examine thyroxine concentrations in plasma (see below for details). In addition, two secondary covert feathers were collected to quantify corticosterone concentrations as a measure of stress over time in herring gull chicks in 2011 and 2012 and in common tern chicks in 2011 (see below). Chicks were examined for morphological deformities during the post-hatch visit in 2011 and when chicks were $\geq$ 21 days old in 2012. An opportunistic deformity survey was also performed in 2011 on as many chicks as possible from nests outside of the enclosures.

Due to the general unpredictability of common terns during the nesting season (e.g., lack of fidelity to nesting colonies), there were challenges associated with determining productivity using the methods reported above. This was notably evident following abandonment of the Hay Point common tern colony early in the breeding season of 2011 where there was evidence of significant predation of tern eggs. Given these challenges, only one visit was made to Hay Point, North Sister Rock, and Cousins Island in late May for the purpose of collecting freshly-laid eggs for artificial incubation in the laboratory in 2012.

As part of a separate EC study of breeding site tenacity and productivity of common terns on the North Channel, productivity estimates are available for colonies at North Sister Rock and Cousins Island in 2011 and 2012 (D. Moore and D.V. Weseloh, EC unpublished) which will be reported here. In addition, egg measurements for 3-egg clutches of common terns at study sites were collected as part of this intensive EC study and will be included here. In 2011 and 2012, eggs were also measured at one additional AOC tern colony on South Sister Rock (46°18'N, 83°55'W) which is adjacent to North Sister Rock and hence just outside of the AOC boundary.

Artificial Incubation of Eggs:

Unincubated eggs were collected in the field, transported to NWRC in insulated coolers with foam inserts, gently cleaned and placed in a Petersime incubator (model# MX-1) at 37^{o}C, 58% humidity and turned every two hours. Just prior to the pipping stage of development (i.e., embryonic day 26-27 for herring gulls and day 20-21 for common terns), embryos were removed from their shells and euthanized by decapitation. Each embryo was examined for physical deformities. Embryonic viability was determined as the number of viable embryos that survived to the designated embryonic day (i.e., just prior to pipping) divided by the total number of fertile eggs. Eggs that were nonviable were staged if possible (e.g., infertile; early, mid or late embryo death). Egg contents, including yolk sac, whole carcass and shell membranes, were collected in chemically-cleaned glass jars and frozen until chemical analysis for contaminants. Ten embryos were randomly selected from each colony for chemical analysis in the two study years. The one exception to this was at the Hay Point tern colony in 2011 where twenty embryos were randomly selected for analysis. This occurred because a second AOC tern colony was not available in that year for comparison purposes.

Contaminant Analyses:

Chemical analyses of individual herring gull and common tern embryos for organochlorine compounds, polybrominated diphenyl ethers (PBDEs), and mercury were conducted at NWRC. Organochlorine compounds measured included *p,p'*-DDE (dichlorodiphenyldichloroethylene), *p,p'*-DDT (dichlorodiphenyltrichloroethane), *p,p'*-DDD (dichlorodiphenyldichloroethane), oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, hexachlorobenzene (HCB), dieldrin, heptachlor epoxide (HE), octachlorostyrene (OCS), mirex, and PCBs. Sum chlordane is based on the sum concentrations of oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, and *trans*-nonachlor. Prior to chemical analysis, thawed embryos were homogenized and then underwent neutral extraction and removal of lipids and biogenic compounds by gel permeation chromatography and Florisil column chromatography. Quantitative analysis of organochlorine compounds was performed using gas chromatography-mass selective detection (GC/MSD) operated in selected ion monitoring mode. The first and second injections were for the determinations of organochlorine pesticides and PCBs, respectively, and the third injection using GC/MSD chemical analysis in the NICI mode was for analysis of PBDEs. Sum PCBs and sum PBDEs were based on the sum concentrations of 62 individual or co-eluting PCB congeners and 15 PBDE congeners found above the limit of detection. Double-crested cormorant (*Phalacrocorax auritus*) egg reference material and two additional certified fish reference materials, blanks and duplicate samples were also analyzed for quality assurance purposes. Concentrations of

organochlorines and PBDEs are reported in µg/g on a wet weight basis. The detection limit for both organochlorine compounds and PBDEs was 0.0001 µg/g.

Embryos were also analyzed for non-*ortho* substituted PCBs, polychlorinated dibenzo-*p*-dioxins, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and polychlorinated dibenzofurans using gas chromatography-high resolution mass spectrometry (GC/HRMS) at RPC Laboratory in Fredericton, New Brunswick. Methods were based on US EPA Method 1613B and 8290A for dioxins and furans and on US EPA Method 1668C for non-*ortho* PCBs. Reference materials, blanks, and duplicates were also analyzed for quality assurance purposes. Following concerns regarding potential effects associated with elevated exposure to dioxin-like PCBs, dioxins and furans in birds in the AOC, deformed and normal embryos from St. Marys River AOC colonies were analyzed separately. A total of five deformed herring gull and common tern embryos from AOC colonies in 2011 and 2012 were analyzed as individuals. Contaminant concentrations in these individuals were then compared to concentrations in normal embryos from AOC colonies (analyzed as 2 or 3 pools) and each of the reference colonies (analyzed as a single pool). All pools consisted of five individual embryos.

Pooled samples of herring gull embryos were analyzed for polycyclic aromatic hydrocarbons (PAHs) at Axys Analytical Services Ltd. in Sidney, British Columbia. Individual pools, consisting of 15 gull embryos collected in 2012, were analyzed for PAHs from the two St. Marys AOC colonies (Hay Point and Pumpkin Point) and the reference colony (Double Island). Quantification of PAHs was performed by low-resolution mass spectrometry (LRMS) using an RTX-5 capillary GC column based on AXYS METHOD MLA-021 Rev 12 and determined by multi-point calibration. A total of 76 PAH and biphenyl compounds were screened for including 20 parent PAH compounds and 56 alkylated PAH (either specific compounds or groups) and biphenyl compounds. Concentrations were blank-corrected and compounds with concentrations that were less than three times the concentration of the lab blank were considered not quantifiable. Sample detection limits for all compounds ranged from 0.03 to 0.25 ng/g. Compounds that were not quantifiable or were below sample detection limits were given a zero value for calculating sum PAH concentrations. Concentrations of PAHs in embryo pools are reported in ng/g on a wet weight basis.

Total mercury was quantified on a dry weight basis using an Advanced Mercury Analyzer (AMA-254) as described in CWS Method No. MET-CHEM-AA-03I. Certified reference materials and duplicate samples were also analyzed to ensure correct calibration, accuracy and reproducibility of test methods. Mercury concentrations in 2011 and 2012 embryos are reported in µg/g on a wet weight basis using percent moisture content.

Stable Isotopes:

Stable isotope analyses of embryos were conducted at the University of Ottawa's G.G. Hatch Stable Isotope Laboratory in Ottawa, Ontario. Samples were weighed into tin capsules and loaded into an elemental analyser. The sample was flash combusted at about 1800^{o}C (Dumas combustion) and the resultant gas products carried by helium through columns of oxidizing/reducing chemicals optimised for CO_2 and N_2. The gases were separated by a purge and trap adsorption column and then sent to the Delta Advantage isotope ratio mass spectrometer coupled with Conflo III. Samples were normalized to

internal standards and calibrated to international standards. Stable isotope ratios are expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following relationship:

$$\delta X = (R_{sample}-R_{standard})/R_{standard} \times 1000$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. In this study, $\delta^{15}N$ signatures were compared to infer relative (and not absolute trophic) position at colonies.

Fatty acid analyses of embryos were also conducted to assess diet composition of egg-laying birds. These results are not yet available and, as such, will not be presented at this time.

Feather Corticosterone:

Feathers from chicks were prepared by first removing the calamus, i.e., the proximal end of the quill to where the feathers start (~ 5 to 10 mm; depending on length of feather). The remaining portion was then minced with scissors until homogenous and 1.5 ml methanol was added. The sample was vortexed, sonicated for 30 minutes at room temperature, and incubated overnight at 50^{o}C while shaking. After centrifuging at 13,000 rpm for 20 minutes, the supernatant was transferred to a fresh tube and re-extracted with 1 ml methanol. The sample was again vortexed for 5 minutes and 0.5 ml of supernatant removed and added to the previous fraction. The methanol fraction was evaporated to dryness overnight in a fume hood and then the sample was reconstituted with 200 µl steroid diluent.

Corticosterone concentrations in the extracted feather sample were determined using a corticosterone EIA kit (Assay Designs – corticosterone enzyme immunoassay kit; product no. 900-097; 96 well kit). This is a competitive immunoassay for the quantitative determination of corticosterone in biological fluids. The kit uses a polyclonal antibody to corticosterone to bind in a competitive manner. Corticosterone in the standard or sample or an alkaline phosphatase molecule which has corticosterone covalently attached to it can be measured.

After a simultaneous incubation at room temperature, the excess reagents were washed away and substrate was added. After a short incubation time, the enzyme reaction was stopped and the sample was read on a SpectraMax 190 UV-VIS microplate reader (Molecular Devices, Sunnyvale, California, USA) at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of corticosterone in either standards or samples. The measured optical density was used to calculate the concentration of corticosterone. The average net optical density (OD) bound for each standard and sample is calculated by subtracting the average NSB (non-specific binding) OD from the average OD bound:

Average Net OD = Average Bound OD - Average NSB OD

Then the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo) was calculated using the following formula:

Percent Bound = Net OD x 100 Net Bo OD

The plot of percent bound versus concentration of corticosterone for the standards was graphed using Prism software (GraphPad, La Jolla, California, USA) and a line fitted through the points. The concentration of corticosterone in the unknowns was then determined. An in-house quality control sample was used in each plate. The sensitivity of the method was 27 pg/ml.

Thyroid Status:

Blood samples were centrifuged for 5 minutes at 14,000 rpm to separate plasma from red blood cells. Plasma was stored at -80°C and red blood cells were stored at 4°C. Concentrations of free thyroxine in the plasma of chicks sampled in 2012 were determined using a commercially available kit (AccuBind Elisa microwells product no. 1225-300 - Monobind Inc., Lake Forest, CA, 92630, USA). The method is based on a competitive enzyme immunoassay format in which a competition is set up between an immobilized antibody, the enzyme-antigen conjugate and the free thyroxine in the sample. When equilibration is reached, the unbound antigen fraction is removed and the enzyme activity in the bound fraction is measured which is inversely proportional to the concentration of free thyroxine in the sample. As per the method, standards, controls and sample plasma, as well as the enzyme reagent, were added to the microplate wells, which contained the immobilized antibody. After an incubation period, the unbound fraction was washed from the wells and the substrate added. The reaction was stopped and the plate was read at 450nm on a SpectraMax 190 UV-VIS microplate reader (Molecular Devices, Sunnyvale, California, USA). Concentrations were determined from the standard curve which was fitted using a variable slope (four parameter) method using Prism software (GraphPad, La Jolla, California, USA).

Controls used were Randox product no. HS2611 assayed human sera levels 2 & 3 (Randox Laboratories Ltd., Antrim, UK). The method gave the intra-assay precision as 10.98, 4.26 and 3.25 % coefficient of variation for low, medium and high controls, respectively. The inter-assay precision was 10.81, 6.01 and 7.90 % coefficient of variation for low, medium and high controls, respectively. The sensitivity of the assay was 0.05 ng/dl.

Thyroxine was also quantified in plasma of herring gull and common tern chicks sampled in 2011 using a different type of kit (radioimmunoassay) and, as a result, is not directly comparable to concentrations determined using the method outlined above for 2012 samples. Thyroxine was also quantified in plasma of embryos from artificially incubated eggs in 2011 but was found at concentrations below the limit of detection in all samples.

Statistical Analysis:

Contaminants and other biological endpoints were statistically analyzed using either the Student's *t*-test for between-colony comparisons or a one-way ANOVA for among-colony comparisons, which when significant, was followed by Tukey's HSD test. Data were log-transformed (log_{10}) to meet conditions of equal variance and normality for parametric analysis. If data failed these assumptions, comparisons were made using either a Mann-Whitney U non-parametric test or Kruskal-Wallis one way analysis of variance by ranks; post-hoc tests were conducted using non-parametric multiple comparison tests for unequal sample sizes. Concentrations of contaminants found below the limit of detection were given a concentration of one-half of the detection limit. While mercury concentrations in embryos were

statistically analyzed on a dry weight basis, these are reported on a wet weight basis for comparisons to published values and thresholds. A Spearman rank correlation analysis was performed to examine the relationship between the two stable isotopes for each species. Due to the few number of deformed embryos found, the Fisher exact test (one-tailed) was used to compare counts of deformed and non-deformed embryos at AOC colonies and reference colonies which were combined in the two study years. All results were considered significant at $p<0.05$.

Concentrations of 2,3,7,8-TCDD toxic equivalents (TEQs) were calculated for dioxin-like PCBs, furans, and dioxins and are based upon toxic equivalency factors developed by van den Berg *et al.* (1998) for birds. Dioxin-like PCBs include four non-*ortho* PCB congeners (77, 81, 126, and 169) and eight mono-*ortho* PCB congeners (105, 114, 118, 123, 156, 157, 167, and 189). Two of these eight mono-*ortho* PCBs (123 and 167) were not quantified as individual congeners in this study and were not included in the calculation of TEQs. For normal embryos analyzed as pools, mean mono-*ortho* PCB concentrations, quantified in the chemical analysis for organochlorines, were calculated for individuals used to create the pool (where data were available). Due to the random sampling of individuals selected for organochlorine analysis, mono-*ortho* PCB concentrations were not available for one deformed herring gull embryo from Hay Point in 2011. Total TEQ concentration is based on the sum concentration of TEQs calculated for the 4 non-*ortho* PCBs, 6 mono-*ortho* PCBs, and 17 dioxin and furan congeners. Concentrations were compared between deformed embryos and normal embryos of herring gulls from AOC colonies in 2011 and 2012 using a Student's *t*-test; a similar statistical comparison was not possible for common terns due to low sample size (N=2).

RESULTS

A) Artificial Egg Incubation Study

Embryonic Viability and Deformities:

i) Herring gulls

Embryonic viability was high in herring gulls from the two AOC colonies, Pumpkin Point and Hay Point, and ranged between 92%-100% in 2011 and 2012 (Table 1a). Embryonic viability was also high at the Double Island reference colony at 86% and 96% in 2011 and 2012, respectively. Of 52 fertile eggs examined in 2011, a total of five embryos died: one from Pumpkin Point (at developmental stage 26), one from Hay Point (stage 39) and three from Double Island (stages 19, 35, and 39). Of 50 fertile eggs examined in 2012, two embryos died, one from Pumpkin Point (stage 31) and one from Double Island (stage 29). Embryonic deformities were evident in a single embryo from both AOC colonies in at least one study year. Incidences of deformities at Pumpkin Point were 6% (1 deformed embryo/17 fertile embryos) in 2011 and 8% (1/13) in 2012 and at Hay Point were 7% (1/14) in 2011 and 0% (0/12) in 2012. Of the two deformed embryos from Pumpkin Point in 2011 and 2012, one had a crossed-bill and part of the lower brain was exposed while the other individual had a split lower mandible. The one deformed embryo from Hay Point had a slightly off-centre lower mandible. No embryonic deformities were evident in incubated eggs from Double Island in either study year. No significant difference was found in the incidence of herring gull embryonic deformities between AOC colonies and the reference colony when counts for sites and years were combined.

Table 1. Embryonic viability and incidence of embryonic deformities in artificially-incubated eggs of herring gulls (a) and common terns (b) collected from St. Marys River AOC colonies (Hay Point, Pumpkin Point, North Sister Rock) and corresponding Lake Huron reference colonies (Double Island or Cousins Island) in 2011 and 2012.

a) Herring gulls:

Colony	AOC/ Ref	Year	Total No. Eggs	No. Infertile Eggs	No. Fertile Eggs	No. Viable Eggs	No. Dead Eggs	Embryonic Viability (%)	No. Deformities	Deformities (%)
Hay Point	AOC	2011	15	1	14	13	1	93%	1	7%
		2012	15	3	12	12	0	100%	0	0%
Pumpkin Point	AOC	2011	17	0	17	16	1	94%	1	6%
		2012	15	2	13	12	1	92%	1	8%
Double I.	Ref	2011	23	2	21	18	3	86%	0	0%
		2012	26	1	25	24	1	96%	0	0%

b) Common terns:

Site	AOC/ Ref	Year	Total No. Eggs	No. Infertile Eggs	No. Fertile Eggs	No. Viable Eggs	No. Dead Eggs	Embryonic Viability (%)	No. Deformities	Deformities (%)
Hay Point	AOC	2011	30	1	29	26	3	90%	1	3%
		2012	15	2	13	13	0	100%	0	0%
North Sister Rock	AOC	2012	15	1	14	13	1	93%	1	7%
Cousins I.	Ref	2011	15	0	15	14	1	93%	0	0%
		2012	15	5	10	10	0	100%	0	0%

ii) Common terns

Embryonic viability among artificially-incubated common tern eggs from the two AOC colonies, Hay Point and North Sister Rock, in 2011 and 2012 was high and ranged between 90%-100% (Table 1b). Embryonic viability at the Cousins Island reference colony was comparable at 93% in 2011 and 100% in 2012. Of the 44 fertile eggs examined in 2011, four embryos died: three from Hay Point (stages 6, 8, and 17) and one from Cousins Island (stage 17). Of the 37 fertile eggs examined in 2012, one embryo from North Sister died (stage 25). Similar to herring gulls, embryonic deformities were evident in a single embryo from each of the two AOC colonies in one study year. Incidences of embryonic deformities were 3% (1/29) at Hay Point in 2011 and 7% (1/14) at North Sister Rock in 2012. The deformed embryo from Hay Point had one eye, half a skull and the lower body was shortened while the deformed individual from North Sister Rock had long thin limbs. No embryonic deformities were evident in incubated eggs from Cousins Island in either study year. No significant difference was found in the incidence of common tern embryonic deformities between AOC colonies and the reference colony when counts for sites and years were combined.

Contaminants:

i) Herring gulls

Concentrations of organochlorine compounds were low overall in herring gull embryos from the two AOC colonies and the reference colony in 2011 and 2012 with sum PCBs found at the highest concentrations (Table 2). Mean sum PCB concentrations ranged from 1.13 µg/g in embryos from Double Island in 2012 to 1.92 µg/g in embryos from Pumpkin Point in 2011. The maximum sum PCB concentration of 4.88 µg/g was found in an embryo from Double Island in 2011. Mean concentrations of the remaining organochlorines in embryos were below 0.4 µg/g at the three colonies. Sum PBDE concentrations (determined as the sum concentration of 15 PBDE congeners) were comparable to concentrations of *p,p'*-DDE in embryos and, based on comparisons of means, were at least six times higher than concentrations of other organochlorine pesticides. The maximum sum PBDE concentration (1.01 µg/g) was found in an embryo from Pumpkin Point in 2012. Generally, no significant differences in concentrations of organochlorines, including sum PCBs, and sum PBDEs were found in herring gull embryos among the three colonies in either 2011 or 2012. The one exception was for octachlorostyrene (OCS) in 2011 in which a significantly higher mean concentration was found in embryos from Pumpkin Point compared to those from the Hay Point AOC colony and Double Island reference colony. Concentrations of this compound, however, were among the lowest of all measured in this study. Mean percent lipid content was not significantly different among colonies within a study year and ranged from 6.5% in herring gull embryos from Double Island in 2011 to 8.4% in embryos from Pumpkin Point in 2012.

Mean concentrations of four non-*ortho* PCBs, 2,3,7,8-TCDD, and total TEQs were largely comparable among deformed and normal herring gull embryos from St. Marys River AOC colonies and the Double Island reference colony in 2011 and 2012 (Table 3a). Of the four non-*ortho* PCBs measured in deformed herring gull embryos, concentrations of PCB-126 > 77 > 81 ≈ 169. Patterns in normal embryos from AOC colonies and the reference colony were slightly different with concentrations of PCB-126 > 169 > 81 ≈ 77. The mean 2,3,7,8-TCDD concentration in three deformed embryos (4.76 pg/g) was within the range

Table 2. Mean concentrations (SD) of organochlorines and sum PBDEs in herring gull embryos (wet weights) collected from the St. Marys River AOC (Hay Point and Pumpkin Point) and Double Island in Lake Huron (reference colony) in 2011 and 2012 and incubated in the lab. All compounds are shown in µg/g. N values represent number of individual embryos analyzed. Different uppercase letters show significant differences in mean concentrations between colonies within a given year based on a one-way ANOVA.

Colony	AOC/ Ref	Year	*p,p'*-DDE	Sum Chlordane	HCB	Dieldrin	HE	Mirex	OCS	PCB 1:1[1]	Sum PCBs[2]	Sum PBDEs[3]
Hay Point N=10	AOC	2011	0.376 (0.173)	0.037 (0.013)	0.015 (0.005)	0.024 (0.012)	0.013 (0.004)	0.026 (0.034)	0.002 (0.001) **B**	3.435 (1.569)	1.807 (0.842)	0.244 (0.093)
Pumpkin Point N=10	AOC	2011	0.304 (0.092)	0.036 (0.007)	0.018 (0.008)	0.022 (0.009)	0.012 (0.002)	0.013 (0.018)	0.004 (0.001) **A**	3.036 (1.219)	1.922 (0.773)	0.237 (0.094)
Double I. N=10	Ref	2011	0.324 (0.127)	0.031 (0.010)	0.015 (0.006)	0.019 (0.016)	0.011 (0.004)	0.033 (0.038)	0.002 (0.001) **B**	3.196 (2.218)	1.766 (1.338)	0.311 (0.198)
Hay Point N=10	AOC	2012	0.248 (0.125)	0.035 (0.025)	0.009 (0.005)	0.009 (0.007)	0.012 (0.015)	0.010 (0.009)	0.002 (0.003)	2.824 (1.181)	1.433 (0.671)	0.333 (0.147)
Pumpkin Point N=10	AOC	2012	0.292 (0.111)	0.029 (0.007)	0.008 (0.003)	0.010 (0.006)	0.008 (0.004)	0.020 (0.022)	0.001 (0.001)	2.359 (1.007)	1.273 (0.728)	0.452 (0.258)
Double I. N=10	Ref	2012	0.218 (0.048)	0.025 (0.005)	0.008 (0.005)	0.007 (0.005)	0.006 (0.001)	0.010 (0.013)	0.002 (0.002)	2.187 (1.177)	1.131 (0.704)	0.332 (0.185)

[1] Based on 1:1 ratio of Arochlor 1254:1260

[2] Based on the sum of 62 PCB congeners

[3] Based on the sum of 15 PBDE congeners

Table 3. Mean concentrations (SD) of non-*ortho* PCBs, 2,3,7,8-TCDD, and 2,3,7,8-TCDD toxic equivalents (TEQ) in embryos of herring gulls (a) and common terns (b) collected from the St. Marys River (SMR) AOC and reference colonies (i.e., Double Island and Cousins Island) in 2011 and 2012 and incubated in the lab (pg/g, wet weight). Concentrations are shown for deformed embryos (analyzed as individuals) and normal embryos based on the analysis of pools consisting of five individuals. Accordingly, N represents either the number of deformed individuals or the number of pools analyzed. TEQs associated with 4 non-*ortho* PCBs, 17 dioxins and furans (PCDD/Fs), and 6 mono-*ortho* PCBs and which together comprise total TEQs are also provided.

a) Herring gulls:

Colony	AOC/ Ref	N	PCB-77	PCB-81	PCB-126	PCB-169	2,3,7,8 – TCDD	TEQ – non-*ortho* PCBs	TEQ – PCDD/Fs	TEQ – mono-*ortho* PCBs[1]	Total TEQs
SMR[2]- Deformed	AOC	3	249.07 (384.48)	120.10 (53.96)	699.00 (149.79)	112.70 (54.67)	4.76 (0.51)	94.48 (36.38)	12.93 (5.10)	8.03 (0.82)	112.76 (42.36)
SMR[2]- Normal	AOC	3	61.90 (71.18)	72.30 (9.52)	497.67 (70.12)	92.07 (20.57)	3.30 (1.30)	60.18 (10.78)	9.31 (4.21)	7.30 (0.96)	76.78 (15.84)
Double I. - Normal	Ref	1	36.70	98.90	680.00	121.00	5.62	79.85	13.89	9.73	103.46

b) Common terns:

Colony	AOC/ Ref	N	PCB-77	PCB-81	PCB-126	PCB-169	2,3,7,8 – TCDD	TEQ – non-*ortho* PCBs	TEQ – PCDD/Fs	TEQ – mono-*ortho* PCBs	Total TEQs
SMR[3]- Deformed	AOC	2	617.25 (135.41)	55.58 (25.49)	389.75 (259.15)	68.03 (37.16)	2.49 (1.84)	75.46 (35.27)	19.19 (11.76)	3.33 (1.94)	97.98 (48.96)
SMR[3]- Normal	AOC	2	548.50 (9.19)	48.05 (5.73)	346.00 (82.02)	70.20 (18.95)	2.14 (0.64)	66.90 (8.33)	16.09 (2.61)	3.35 (0.60)	86.34 (6.32)
Cousins I. - Normal	Ref	1	1000.00	75.80	455.00	85.20	3.80	103.17	33.24	2.73	139.14

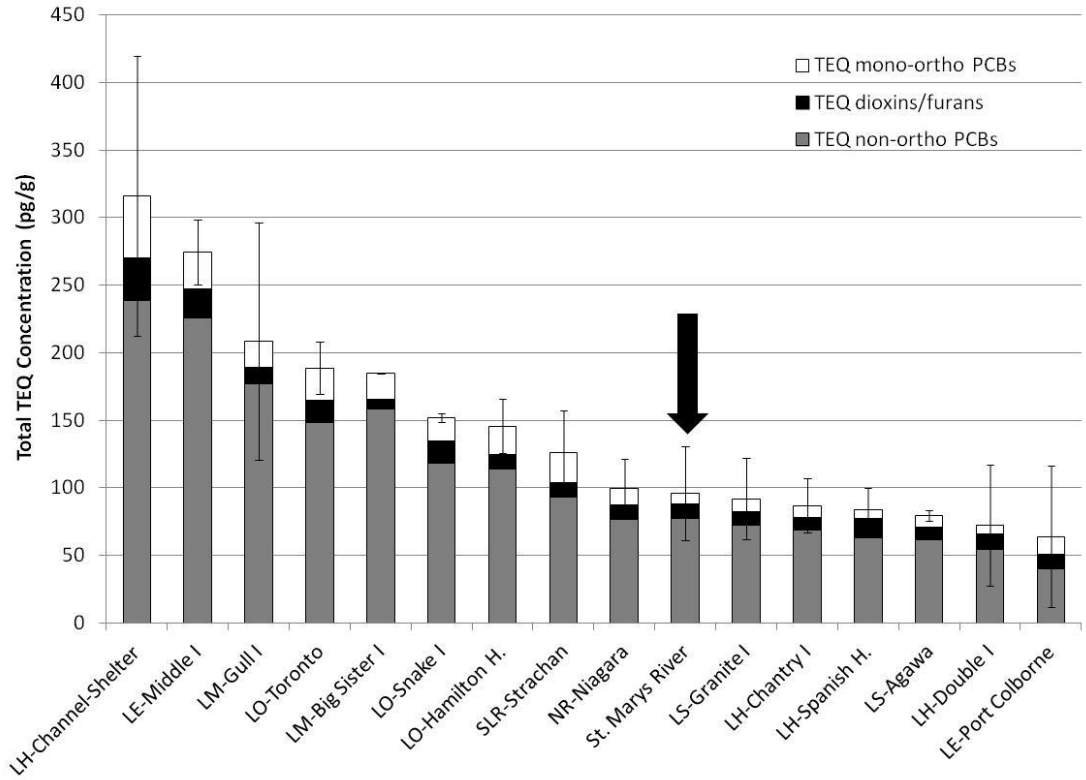
[1] Since TEQ–mono-*ortho* PCBs were quantified in individual embryos (as part of the sum PCB analysis), these concentrations were determined using the mean concentration of individuals which comprised the pool (where data were available with a range of between 1-5 individuals); the mean TEQ–mono-*ortho* PCB concentration for deformed herring gull embryos from SMR is based on N=2 rather than N=3 (see methods for details).

[2] Comprise individuals from Hay Point and Pumpkin Point

[3] Comprise individuals from Hay Point and North Sister Rock

of concentrations found for normal embryos from AOC colonies (3.30 pg/g) and normal embryos from the reference colony (5.62 pg/g) which was also the maximum concentration found in this study. Mean total TEQ concentrations ranged from 76.78 pg TEQ/g in normal embryos from AOC colonies to 112.76 pg TEQ/g in deformed embryos from AOC colonies. The maximum total TEQ concentration (159.17 pg TEQ/g) was found in a deformed embryo from Hay Point in 2011. Toxicity associated with non-*ortho* PCBs, dioxins and furans, and mono-*ortho* PCBs contributed 84%, 11%, and 7%, respectively, to the mean total TEQ concentration in deformed embryos from AOC colonies in 2011 and 2012. Similar contributions of toxicity associated with non-*ortho* PCBs, dioxins and furans, and mono-*ortho* PCBs to mean total TEQ concentrations were found for normal embryos from AOC colonies (78%, 12%, 10%, respectively) and the reference colony (77%, 13%, and 9%, respectively). Concentrations of non-*ortho* PCBs, 2,3,7,8-TCDD, and TEQs were not significantly different between deformed and normal herring gull embryos from AOC colonies ($p>0.05$). Overall, the mean total TEQ concentration in deformed and normal embryos from the St. Marys River AOC (N=6 samples total) was 94.77 pg TEQ/g in 2011 and 2012 and was within the range of mean TEQ concentrations in herring gull eggs from other colonies on the Great Lakes in these two years (Figure 2; EC unpublished).

Figure 2. Mean total TEQ concentrations (SD) in deformed and normal herring gull embryos (N=6 samples total) from St. Marys River AOC (Ontario) colonies and in herring gull eggs from Great Lakes colonies in 2011 and 2012 (pg/g, wet weight). The contributions of TEQ concentrations associated with mono-*ortho* PCBs, dioxins and furans, and non-*ortho* PCBs to the total TEQ concentration are shown. Means are arranged in decreasing order from highest to lowest concentrations. Colony locations are associated with the following lakes/rivers: LH=Lake Huron, LO=Lake Ontario, LE=Lake Erie, SLR=St. Lawrence River, NR=Niagara River, LS=Lake Superior, and LM=Lake Michigan.



A full listing of mean concentrations of non-*ortho* PCBs, mono-*ortho* PCBs, dioxins and furans in deformed and/or normal herring gull embryos from St. Marys River AOC colonies and the Double Island reference colony is provided in Appendix 1.

Sum PAH concentrations in single pools of herring gull embryos from the two AOC colonies in 2012 were 21.64 ng/g at Hay Point and 13.02 ng/g at Pumpkin Point while the sum PAH concentration in the single pooled sample from the Double Island reference colony was similar to that from Pumpkin Point at 13.52 ng/g (Table 4). Four parent PAH compounds, anthracene, benz[a]anthracene, benzo[b]-fluoranthene, and benzo[j,k]-fluoranthenes, were detected in embryos in this study; the remaining 16 parent PAHs were not detected. Sum concentrations of parent compounds in embryos ranged from 0.09 ng/g at Pumpkin Point to 0.36 ng/g at Double Island. Sum concentrations of the alkylated PAHs ranged from 12.93 ng/g at Pumpkin Point to 21.31 ng/g at Hay Point. Numbers of alkylated PAHs detected varied among embryos from the three colonies and ranged from 16 compounds in gulls from Double Island to 26 compounds in gulls from Hay Point. Sum concentrations of all PAHs in embryos were largely dominated by the alkylated compounds which comprised 97%-99% of the sum PAH concentration in embryos from the three colonies. Sum concentrations of all PAHs were higher in gulls from Hay Point however it is important to note that these results are based on the analysis of a single pool of embryos from each colony.

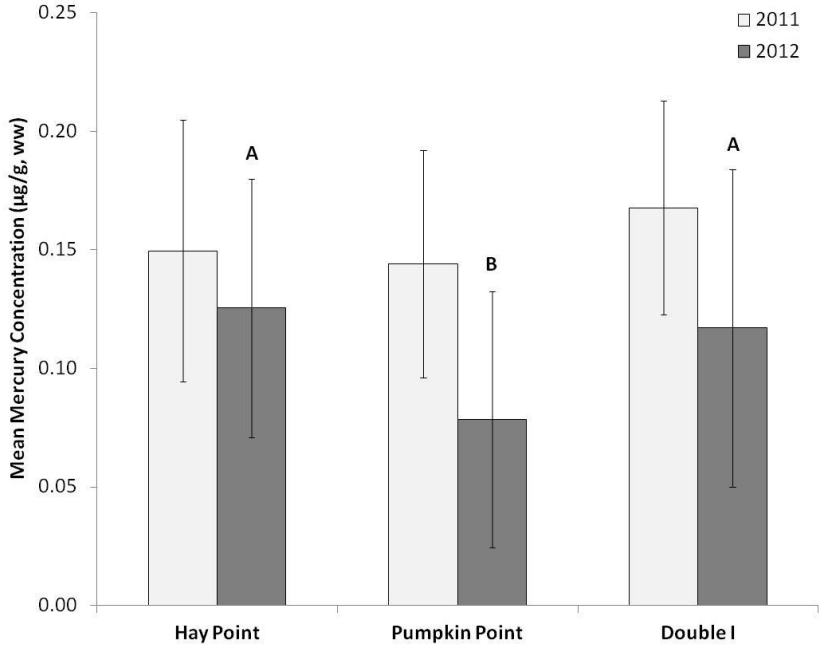
Table 4. Concentrations of PAHs in pools of herring gull embryos collected from the St. Marys River AOC (Hay Point and Pumpkin Point) and Double Island in Lake Huron (reference colony) in 2012 and incubated in the lab. Compounds are shown in ng/g (wet weight). Numbers in brackets represent the number of compounds that were detected. A single pool consisting of 15 embryos was analyzed per colony. "-" denotes that the compound was either not quantifiable or was below the sample detection limit.

Colony	Parent PAH Compounds				Sum Parent PAHs (20)	Sum Alkylated PAHs (56)	Sum All PAHs (76)
	Anthracene	Benz[a]-anthracene	Benzo[b]-fluoranthene	Benzo[j,k]-fluoranthenes			
Hay Point	0.13	-	-	0.20	0.33 (2)	21.31 (26)	21.64 (28)
Pumpkin Point	0.09	-	-	-	0.09 (1)	12.93 (17)	13.02 (18)
Double I.	0.05	0.04	0.27	-	0.36 (3)	13.16 (16)	13.52 (19)

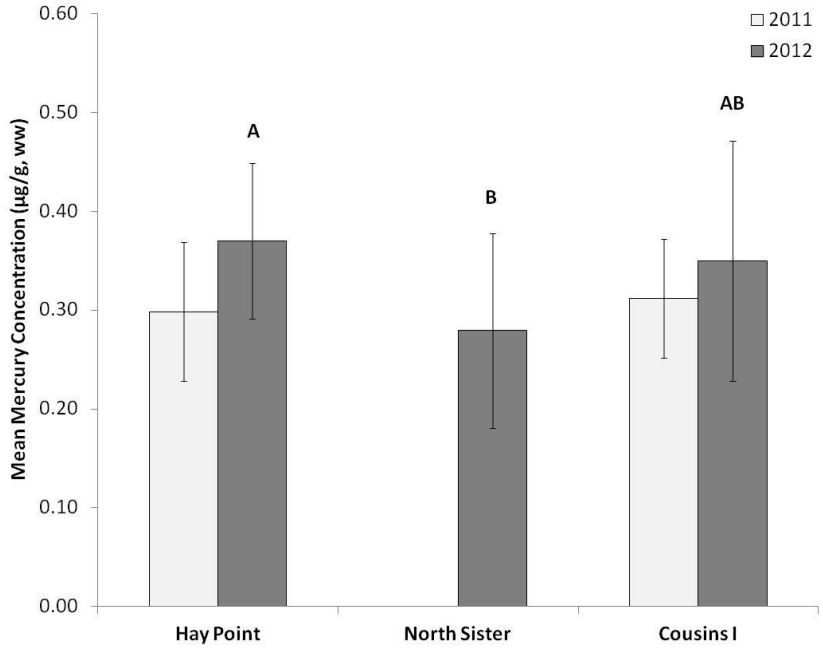
Mean mercury concentrations (SD) in herring gull embryos in 2011 were statistically comparable among the three study sites ranging from 0.14 (0.05) µg/g wet weight at Pumpkin Point to 0.17 (0.05) µg/g at Double Island (Figure 3a). In 2012, some spatial differences among colonies were evident with mean mercury concentrations (SD) ranging from 0.08 (0.05) µg/g at Pumpkin Point to 0.13 (0.05) µg/g at Hay Point. Mercury concentrations in embryos from Hay Point were statistically similar to those from Double Island and embryos from both colonies had significantly higher mercury concentrations than embryos from Pumpkin Point ($F_{2,42}$=5.54, p=0.007). The maximum mercury concentration was 0.28 µg/g in a herring gull embryo from Hay Point in 2011.

Figure 3. Mean concentrations (SD) of total mercury (µg/g, wet weight) in embryos of herring gulls (a) and common terns (b) collected from St. Marys River AOC colonies (Hay Point, Pumpkin Point, North Sister Rock) and corresponding Lake Huron reference colonies (Double Island or Cousins Island) in 2011 and 2012 (N=12-30 embryos/colony). Different uppercase letters show significant differences in mean concentrations within a study year and are based on statistical analysis of dry weight mercury concentrations. Note no data are available for common terns at North Sister Rock in 2011.

a) Herring gulls:



b) Common terns:



ii) Common terns

Contaminant burdens were also very low in common terns from AOC and reference colonies in 2011 and 2012 (Table 5). Sum PCBs were found at the highest concentrations relative to other organochlorines with means ranging from 0.77 µg/g in embryos from Hay Point in 2011 to 1.43 µg/g in embryos from Cousins Island in 2012. The maximum sum PCB concentration reported (2.58 µg/g) was found in an embryo from the Cousins Island reference colony in 2012. Similar to herring gulls, sum PBDEs in common tern embryos at study sites were comparable in concentration to *p,p'*-DDE and higher than concentrations of other organochlorine pesticides. The maximum sum PBDE concentration (0.21 µg/g) was found in an embryo from North Sister Rock in 2012. Some spatial differences were apparent between/among colonies in each of the two study years but in a somewhat unexpected direction. In 2011, significantly higher concentrations of sum PCBs, sum PBDEs, *p,p'*-DDE, sum chlordane and OCS were found in common tern embryos from the Cousins Island reference colony compared to the Hay Point AOC colony. Concentrations of HCB, dieldrin, HE and mirex were comparable between study sites. In 2012, significantly higher concentrations of *p,p'*-DDE, mirex and OCS were found in embryos from the Cousins Island reference colony compared to the Hay Point AOC colony while concentrations were comparable between the reference colony and the North Sister Rock AOC colony. In 2012, concentrations of other contaminants including sum PCBs and sum PBDEs were similar among study sites. Percent lipid content was not significantly different between colonies in 2011 with means in common tern embryos of 6.8% and 7.6% at Hay Point and Cousins Island, respectively. In 2012, percent lipid content was significantly higher in embryos from Cousins Island (mean=9.8%) compared to Hay Point (mean=8.8%; $F_{2,27}$=5.64, p=0.009). As such, the results reported in Table 5 are based on contaminants data in 2012 which were first lipid-normalized prior to statistical analysis.

Mean concentrations of four non-*ortho* PCBs, 2,3,7,8-TCDD, and total TEQs were comparable between deformed and normal common tern embryos from St. Marys River AOC colonies in 2011 and 2012 (Table 3b). Concentrations in tern embryos from the Cousins Island reference colony (analyzed as a pool) were generally slightly higher compared to embryos from AOC colonies and most frequently were the maximum concentration found of all embryos. Of the four non-*ortho* PCBs measured in common tern embryos, concentrations of PCB-77 > 126 > 169 ≈ 81, a pattern which differed to that found in herring gull embryos. The mean 2,3,7,8-TCDD concentration in two deformed embryos (2.49 pg/g) was within the range of concentrations found for normal embryos from AOC colonies (2.14 pg/g) and normal embryos from the reference colony (3.80 pg/g). Mean total TEQ concentrations ranged from 86.34 pg TEQ/g in normal embryos from AOC colonies to 139.14 pg TEQ/g in normal embryos from the reference colony. Toxicity associated with non-*ortho* PCBs, dioxins and furans, and mono-*ortho* PCBs contributed 77%, 20%, and 3%, respectively, to the mean total TEQ concentration in deformed embryos from AOC colonies in 2011 and 2012. Similar contributions of toxicity associated with non-*ortho* PCBs, dioxins and furans, and mono-*ortho* PCBs to mean total TEQ concentrations were found for normal embryos from the AOC and the reference colony. Due to low sample sizes, it was not possible to statistically compare concentrations of non-*ortho* PCBs, 2,3,7,8-TCDD, and TEQs between deformed and normal common tern embryos from AOC colonies. An examination of mean concentrations with associated variation however suggests that concentrations between these two groups are very similar. A full listing of mean concentrations of non-*ortho* PCBs, mono-*ortho* PCBs, dioxins and furans in deformed and/or normal

Table 5. Mean concentrations (SD) of organochlorines and sum PBDEs in common tern embryos (wet weights) collected from the St. Marys River AOC (Hay Point and North Sister Rock) and Cousins Island in Lake Huron (reference site) in 2011 and 2012 and incubated in the lab. All compounds are shown in µg/g. N values represent number of individual embryos analyzed. Different uppercase letters show significant differences in mean concentrations between colonies within a given year based on a one-way ANOVA.

Colony	AOC/ Ref	Year	*p,p'*-DDE	Sum Chlordane	HCB	Dieldrin	HE	Mirex	OCS	PCB 1:1[1]	Sum PCBs[2]	Sum PBDEs[3]
Hay Point N=20	AOC	2011	0.099 (0.038) **B**	0.009 (0.005) **B**	0.011 (0.003)	0.014 (0.005)	0.004 (0.002)	0.004 (0.002)	0.0013 (0.0005) **B**	1.348 (0.590) **B**	0.768 (0.300) **B**	0.065 (0.021) **B**
Cousins I. N=10	Ref	2011	0.178 (0.044) **A**	0.014 (0.005) **A**	0.013 (0.004)	0.016 (0.006)	0.005 (0.002)	0.005 (0.003)	0.0021 (0.0006) **A**	2.116 (0.370) **A**	1.147 (0.165) **A**	0.094 (0.024) **A**
Hay Point N=10	AOC	2012	0.146 (0.061) **B**	0.009 (0.006)	0.013 (0.004)	0.017 (0.006)	0.006 (0.002)	0.003 (0.001) **B**	0.0012 (0.0005) **B**	1.902 (0.703)	0.890 (0.305)	0.090 (0.032)
North Sister N=10	AOC	2012	0.181 (0.046) **AB**	0.012 (0.009)	0.016 (0.004)	0.019 (0.004)	0.006 (0.002)	0.005 (0.002) **A**	0.0016 (0.0004) **AB**	2.474 (0.689)	1.146 (0.311)	0.124 (0.387)
Cousins I. N=10	Ref	2012	0.238 (0.076) **A**	0.015 (0.013)	0.019 (0.005)	0.017 (0.004)	0.005 (0.001)	0.006 (0.002) **A**	0.0020 (0.0007) **A**	3.036 (1.262)	1.430 (0.602)	0.140 (0.032)

[1] Based on 1:1 ratio of Arochlor 1254:1260

[2] Based on the sum of 62 PCB congeners

[3] Based on the sum of 15 PBDE congeners

common tern embryos from St. Marys River AOC colonies and the Cousins Island reference colony is provided in Appendix 1.

Mercury concentrations in common tern embryos in 2011 were comparable at Hay Point and Cousins Island with means (SD) of 0.30 (0.07) µg/g and 0.31 (0.06) µg/g, respectively (Figure 3b). In 2012, mean mercury concentrations (SD) ranged from 0.28 (0.10) µg/g at North Sister Rock to 0.37 (0.08) µg/g at Hay Point and, similar to that found for herring gulls, a significant spatial pattern among colonies was evident in this year ($F_{2,36}$=3.83, p=0.031). While mean mercury concentrations were statistically similar between each of the AOC colonies and the Cousins Island reference colony, mercury concentrations were significantly higher in common terns from Hay Point compared to those from North Sister Rock. The maximum mercury concentration was 0.59 µg/g in a common tern embryo from Cousins Island in 2012.

Stable Isotopes:

i) Herring gulls

Significant spatial differences for mean $\delta^{15}N$ values in herring gull embryos were found among study sites in 2011 ($F_{2,42}$=6.94, p=0.003) and 2012 ($F_{2,42}$=4.97, p=0.013; Table 6a). As an indicator of trophic position, mean $\delta^{15}N$ values were significantly higher in gulls from the Double Island reference colony compared to one or both of the AOC colonies in both study years. In addition, mean $\delta^{13}C$ values in gull embryos from Double Island were also significantly more depleted (i.e., more negative) than mean values in embryos from the two AOC colonies in both 2011 ($F_{2,42}$=23.23, p<0.00001) and 2012 ($F_{2,42}$=11.22, p=0.0001). No significant correlation was found between $\delta^{15}N$ and $\delta^{13}C$ values when herring gull embryos from all colonies and years were grouped together.

Table 6. Mean (SD) values for $\delta^{15}N$ and $\delta^{13}C$ in embryos of herring gulls (a) and common terns (b) collected from St. Marys River AOC colonies (Hay Point, Pumpkin Point, North Sister Rock) and corresponding Lake Huron Double Island reference colonies (Double Island or Cousins Island) in 2011 and 2012 (N=12-30 embryos/colony). Different uppercase letters indicate significant differences between colonies within a study year.

a) Herring gulls:

Colony	AOC/Ref	$\delta^{15}N$		$\delta^{13}C$	
		2011	2012	2011	2012
Hay Point	AOC	9.00 (0.82) **B**	8.61 (0.44) **B**	-20.40 (1.00) **A**	-21.32 (1.11) **A**
Pumpkin Point	AOC	8.94 (0.39) **B**	8.77 (0.47) **AB**	-20.75 (0.87) **A**	-21.49 (1.20) **A**
Double I.	Ref	9.80 (0.82) **A**	9.29 (0.88) **A**	-22.49 (0.82) **B**	-23.05 (1.00) **B**

b) Common terns:

Colony	AOC/Ref	$\delta^{15}N$		$\delta^{13}C$	
		2011	2012	2011	2012
Hay Point	AOC	10.57 (0.61) **B**	10.53 (0.69) **B**	-25.16 (1.09) **A**	-25.61 (0.88) **A**
North Sister Rock	AOC	NA	11.07 (0.56) **B**	NA	-25.32 (1.48) **A**
Cousins I.	Ref	12.12 (0.32) **A**	12.29 (0.33) **A**	-25.95 (1.02) **B**	-26.63 (0.50) **B**

ii) Common terns

Similar spatial patterns for isotopes were found in common tern embryos (Table 6b). Mean $\delta^{15}N$ values were significantly higher in common terns from the Cousins Island reference colony compared to one or both of the AOC colonies in 2011 (Mann Whitney U=450, p<0.00001) and 2012 ($F_{2,36}$=32.63, p<0.00001). Mean $\delta^{13}C$ values in tern embryos from Cousins Island were significantly more depleted (i.e., more negative) than mean values in embryos from the AOC colonies in both 2011 (Mann Whitney U=91, p=0.001) and 2012 (Kruskal Wallis test H=9.92, p=0.007). Unlike that found for herring gulls, a highly significant negative correlation was found between $\delta^{15}N$ and $\delta^{13}C$ values when common tern embryos from all colonies and years were grouped together (r_s=0.72, p<0.00001).

B) Field Study

Egg Size Parameters:

i) Herring gulls

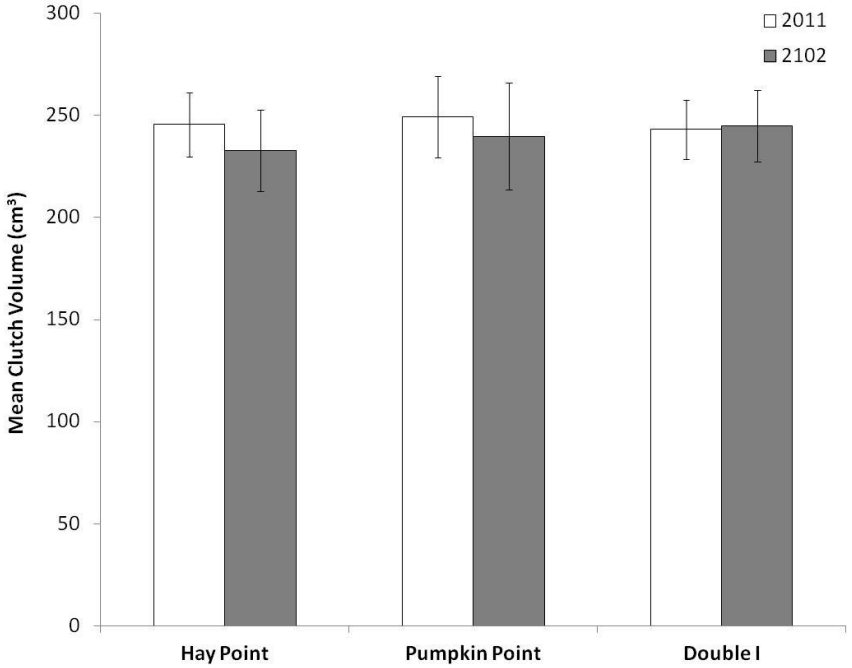
To assess potential food stress in birds, total clutch volume and intraclutch variation in egg size were examined in 3-egg clutches. Mean total clutch volumes (SD) for herring gulls from St. Marys River AOC colonies ranged from 232.8 (19.9) cm^3 at Hay Point in 2012 to 249.2 (19.9) cm^3 at Pumpkin Point in 2011 and overall were not significantly different from total clutch volumes in gulls from the Double Island reference colony in either of the two study years (Figure 4a). Mean intraclutch variation (SD) in egg size from AOC colonies in the two study years ranged from 9.5 (5.5)% at Pumpkin Point in 2011 to 12.0 (7.2)% at Hay Point in 2012 and were also statistically comparable to means (SD) at the Double Island reference colony of 11.1 (3.3)% and 7.3 (4.4)% in each of 2011 and 2012, respectively.

ii) Common terns

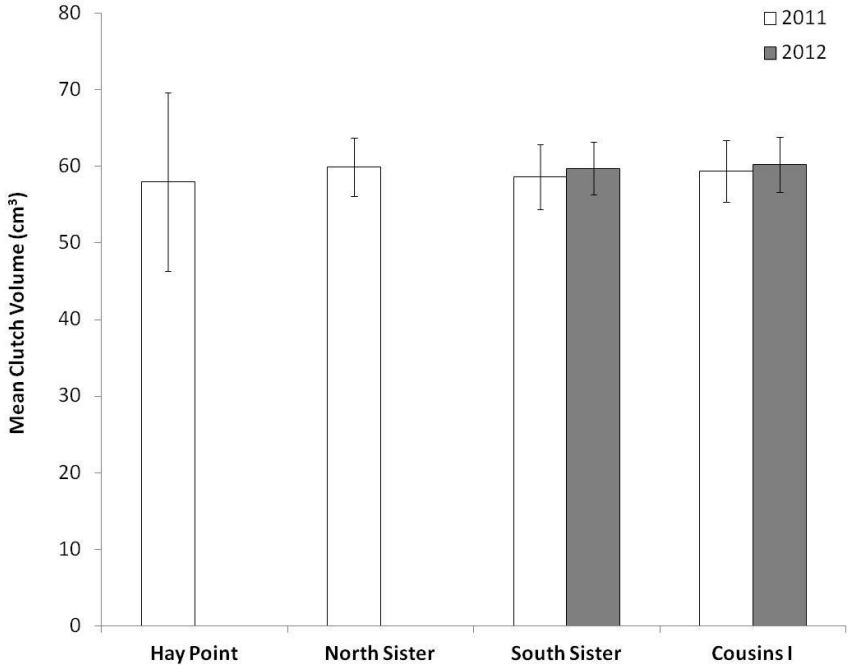
Mean total clutch volume (SD) in 3-egg clutches of common terns at three AOC colonies in 2011 ranged from 58.0 (11.6) cm^3 at Hay Point to 60.0 (3.8) cm^3 at North Sister Rock and were not significantly different from mean clutch volume at Cousins Island (59.4 (4.0) cm^3; Figure 4b). Similarly in 2012, mean total clutch volume (SD) was not significantly different between the South Sister Rock AOC colony (59.8 (3.5) cm^3) and the Cousins Island colony (60.3 (3.6) cm^3). Mean intraclutch variation (SD) in egg size at AOC tern colonies in the two years ranged from 6.8 (3.2)% at South Sister Rock in 2011 to 9.5 (7.6)% at Hay Point in 2011. Despite relatively lower estimates of mean intraclutch variation (SD) in egg size from Cousins Island of 6.2 (2.8)% and 6.0 (2.6)% in 2011 and 2012, respectively, no significant differences in intraclutch variation were found between AOC tern colonies and the reference colony in either of the two study years.

Figure 4. Mean clutch volume (SD) in 3-egg clutches of herring gulls (a) and common terns (b) from St. Marys River AOC colonies (Hay Point, Pumpkin Point, North Sister Rock, South Sister Rock) and corresponding Lake Huron reference colonies (Double Island or Cousins Island) in 2011 and 2012. Numbers of 3-egg clutches ranged from 8-38 for both species with the exception of the Hay Point common tern colony in 2011 where the mean is based on three clutches of eggs. Note no data are available for common terns at Hay Point and North Sister Rock in 2012.

a) Herring gulls:



b) Common terns:

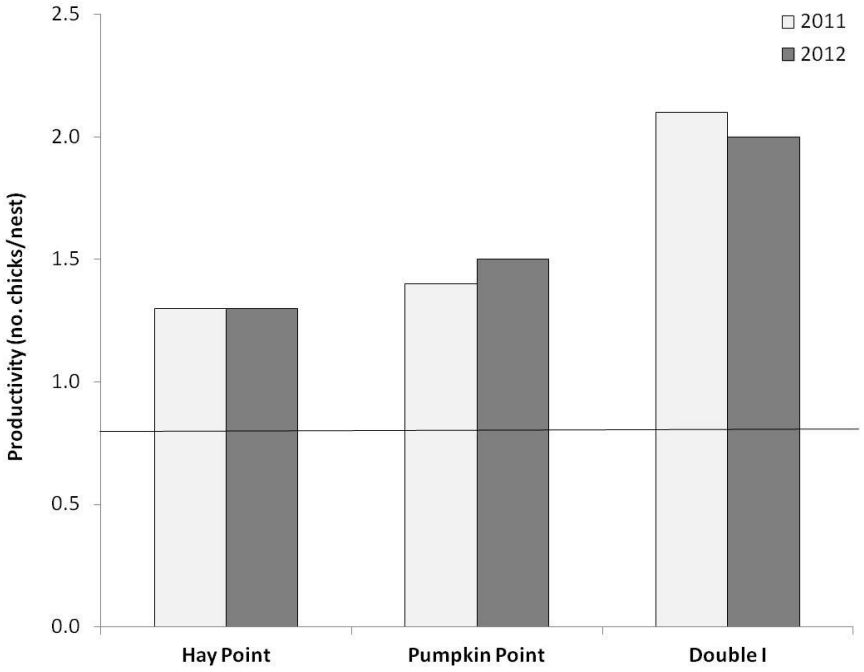


Productivity & Prevalence of Deformities in Wild Populations:

i) Herring gulls

Herring gull productivity, defined as the number of ≥ 21-day-old chicks produced per nest, was equal to 1.3 chicks per nest at Hay Point (N=7 enclosed nests), 1.4 chicks per nest at Pumpkin Point (N=10 nests), and 2.1 chicks per nest at Double Island (N=9 nests) in 2011 (Figure 5). Similarly, productivity was high in 2012 with estimates equal to 1.3 chicks per nest at Hay Point (N=12 nests), 1.5 chicks per nest at Pumpkin Point (N=10 nests), and 2.0 chicks per nest at Double Island (N=7 nests). Productivity estimates at AOC colonies in both study years were well within the range of productivity levels required to maintain a stable population (0.8-1.4 chicks per nest; Kadlec and Drury 1968).

Figure 5. Herring gull productivity, as the number of ≥ 21-day-old chicks produced per nest, at St. Marys River AOC colonies (Hay Point and Pumpkin Point) and the Double Island reference colony in 2011 and 2012. The solid line indicates the minimum productivity level of 0.8 chicks per nest associated with maintaining a stable herring gull population (range in levels=0.8-1.4 chicks per nest; Kadlec and Drury 1968).



In 2011, no morphological deformities were found in herring gull chicks from the two AOC colonies or the reference colony (N=39-76 chicks). This count included chicks from inside and outside of the enclosures (Table 7). Similarly, no deformities were found in gull chicks from enclosed nests at the three colonies in 2012 where numbers of chicks examined ranged from 14-16 per colony.

Table 7. Prevalence of morphological deformities (%) in herring gull chicks examined in enclosed nests and non-enclosed nests from St. Marys River AOC colonies (Hay Point, Pumpkin Point) and the Double Island reference colony in 2011 and 2012.

Colony	Year	No. Chicks Examined		% Deformities
		Enclosed Nests	Non-enclosed Nests	
Hay Point	2011	19	20	0%
	2012	16	-	0%
Pumpkin Point	2011	23	40	0%
	2012	15	-	0%
Double I.	2011	31	45	0%
	2012	14	-	0%

ii) Common terns

Common tern productivity, also defined as the number of $\geq$ 21-day-old chicks produced per nest, was equal to 0.8 chicks per nest at North Sister Rock in 2011 (N=10 enclosed nests; D. Moore and D.V. Weseloh 2012). This productivity estimate was higher than the estimate of 0.3 chicks produced per nest at the Cousins Island reference colony (N=20 nests). In 2012, tern productivity at 15 enclosed nests was equal to 0 at North Sister Rock as a result of predation of eggs in enclosures. There was however some evidence of renesting and successful nests later in the season at this colony (D. Moore, pers. comm.) Although considered low, productivity at Cousins Island was relatively higher in 2012 at 0.6 chicks per nest (N=16 nests). Productivity estimates for all study colonies were well below the threshold of 1.1 common tern chicks per nest associated with a stable population (DiCostanzo 1980).

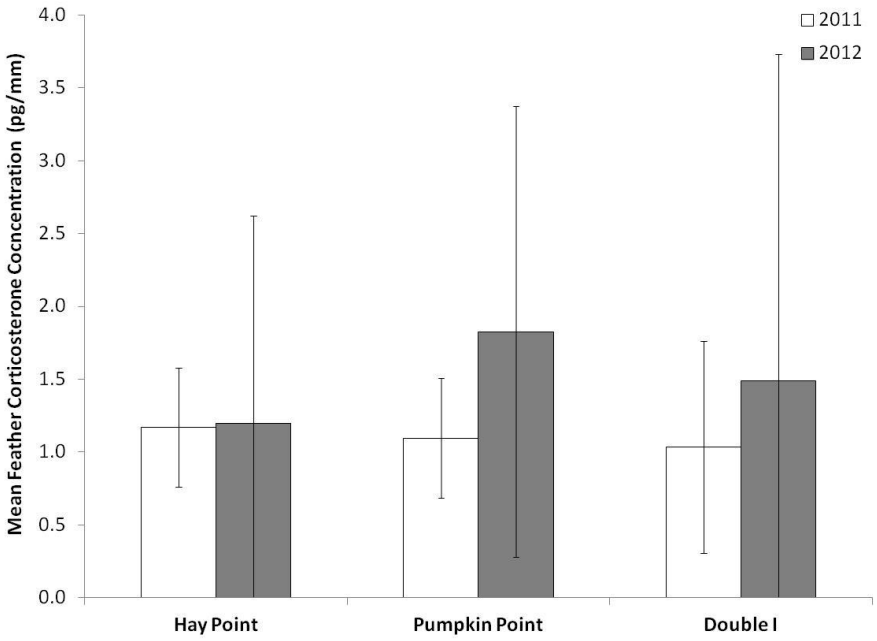
No deformities were observed in common tern chicks examined at non-enclosed nests at North Sister Rock and the Cousins Island reference colony in 2011 (N=13 and 10 chicks respectively). Since common tern colonies were not visited a second time (i.e., when chicks were $\geq$ 21 days old) in 2012, deformity estimates are not available.

Corticosterone in Feathers:

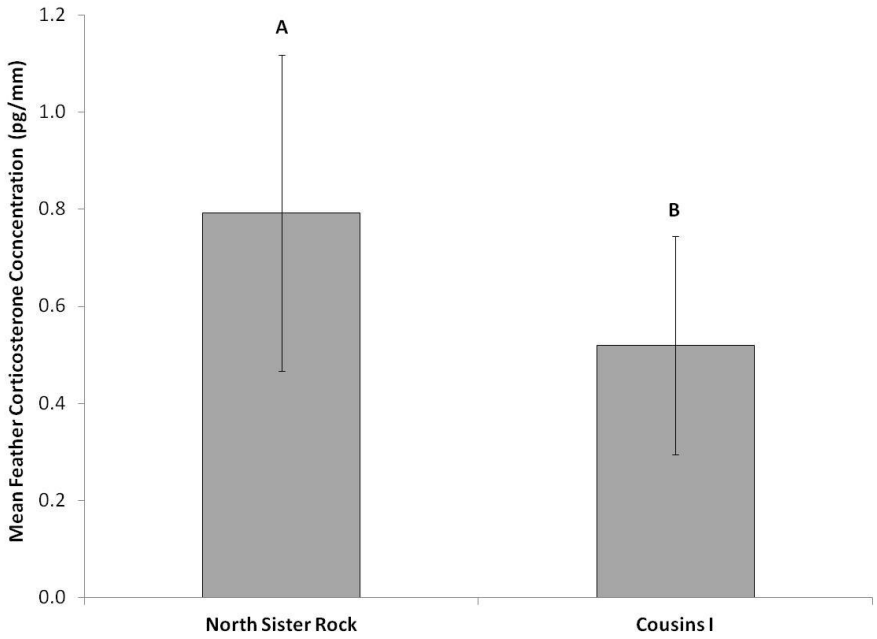
Mean corticosterone concentrations (SD) in herring gull chick feathers from the AOC colonies ranged from 1.1 (0.41) pg/mm to 1.8 (1.6) pg/mm at Pumpkin Point in 2011 and 2012, respectively, and overall were not significantly different from concentrations at the Double Island reference colony in either of the two study years (Figure 6a). However, a significant difference was found for common terns in 2011 (Figure 6b). Chicks from North Sister Rock had a significantly higher mean feather corticosterone concentration compared to those from Cousins Island (t_{21}=2.26; p=0.034). No data are available for common tern chicks in 2012.

Figure 6. Mean corticosterone concentrations (SD), expressed as picograms of corticosterone per millimetre of feather, in chicks of herring gulls (a) and common terns (b) from St. Marys River AOC colonies (Hay Point, Pumpkin Point, North Sister Rock) and corresponding Lake Huron reference colonies (Double Island or Cousins Island) in 2011 and/or 2012. Data for common terns are for 2011 only. Numbers of feathers ranged from 10-20 per colony for both species. Different uppercase letters show significant differences in mean concentrations within a study year.

a) Herring gulls:



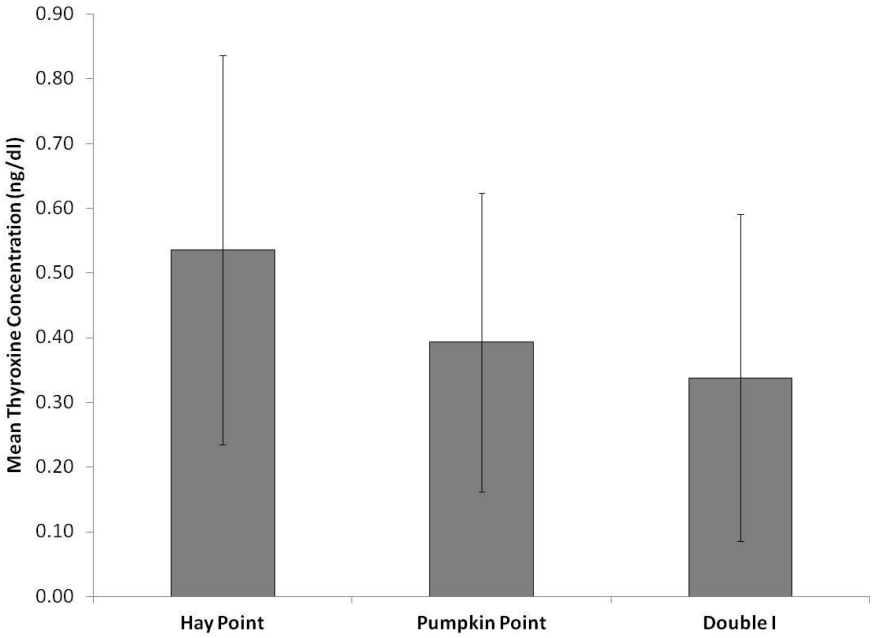
b) Common terns:



Thyroxine in Plasma of Herring Gull Chicks:

Mean thyroxine concentrations (SD) were 0.54 (0.30) ng/dl and 0.39 (0.23) ng/dl in plasma of herring gull chicks from Hay Point and Pumpkin Point, respectively, and 0.34 (0.25) ng/dl in chicks from Double Island in 2012 (Figure 7). No significant difference in mean concentrations was found among colonies. Similarly, no significant differences in thyroxine concentrations were found between chicks from AOC colonies and corresponding reference colonies for herring gulls or common terns in 2011 (data not shown).

Figure 7. Mean thyroxine concentrations (SD) in plasma of herring gull chicks from St. Marys River AOC colonies (Hay Point and Pumpkin Point) and the reference colony (Double Island) in 2012. Numbers of blood samples collected ranged from 13-15 per colony.



DISCUSSION

Based on the low overall contaminant burdens reported in embryos in 2011 and 2012, concentrations of PCBs, other organochlorines and PBDEs were not sufficiently elevated to adversely impact the reproductive success of herring gulls and common terns foraging in the St. Marys River AOC. In a broad literature review of PCB effects in birds, Hoffman *et al.* (1996) concluded that sum PCB concentrations in the range of 8 to 25 µg/g in eggs were associated with decreased hatching success for terns and cormorants. Sum PCB concentrations in all embryos of both species were well below the 8 µg/g threshold. Similarly, concentrations of *p*,*p*'-DDE in eggs were well below threshold levels associated with significant effects on reproductive success as reported in black-crowned night-herons (8 µg/g; Henny *et al.* 1984) and cormorants (10 µg/g; Pearce *et al.* 1979). These findings concur with those found in herring gull eggs from two U.S. colonies in the St. Marys River AOC (Michigan) between 2002-2006

where concentrations of both compounds were below recommended threshold levels associated with adverse reproductive effects (MDEQ 2012). Comparatively, median PCB and *p,p'*-DDE concentrations in gulls from these U.S. colonies were approximately two to three times higher than median concentrations in gulls from the St. Marys River (Ontario) in 2011 and 2012. Sum PBDE concentrations were also low and well below the lowest-observed effect level on pipping and hatching success in American kestrels (*Falco sparverius*) equal to 1.8 µg/g in eggs (McKernan *et al.* 2009). Overall, concentrations of all compounds in both species were largely comparable between the AOC colonies and the reference colonies and were in fact significantly lower for some compounds (e.g., PCBs and PBDEs in terns) at an AOC colony than the reference colony.

Concentrations of 2,3,7,8-TCDD, dioxin-like PCBs and total TEQs in embryos of herring gulls and common terns from the St. Marys River AOC (Ontario) were not notably elevated relative to birds from the respective reference colonies in 2011 and 2012. Concentrations of these compounds in terns from AOC colonies were also below levels found in a previous study of common terns at a colony at Lime Island, on the U.S. side of the St. Marys River, and which is within 1 km of the Hay Point colony (Senthilkumar *et al.* 2003). In that study, ten unhatched eggs collected in 1999 had concentrations of 2,3,7,8-TCDD (range=1.7-6.6 pg/g), dioxin-like PCBs (range in total concentrations of non-*ortho* PCBs and mono-*ortho* PCBs=161-612 ng/g), and total TEQs (91-219 pg TEQ/g) which were considered to be elevated and which also coincided with the collapse of the colony in that year. Tern embryos from AOC colonies in 2011 and 2012 were below concentrations at the Lime Island colony in 1999 with corresponding concentrations of 2,3,7,8-TCDD (range=1.2-3.8 pg/g), dioxin-like PCBs (range=44-110 ng/g), and total TEQs (range=63-133 pg TEQ/g). Median total TEQs in herring gull embryos in this study (85 pg TEQ/g) were less than one-half of median total TEQs (222 pg TEQ/g) in herring gull eggs from colonies on the U.S. side of the St. Marys River between 2002-2006 (MDEQ 2012). Total TEQs in herring gulls from the St. Marys River AOC were within the range of mean TEQ concentrations in herring gull eggs from other colonies on the Great Lakes in 2011 and 2012 (Figure 2).

With respect to concentrations associated with toxicity, concentrations of 2,3,7,8-TCDD (<6 pg/g) and total TEQs (<160 pg TEQ/g) in herring gull and common tern embryos from colonies in the St. Marys River AOC were below concentrations associated with effects on reproduction in avian species. Median concentrations of 2,3,7,8-TCDD (37 pg/g) and total TEQs (2175 pg TEQ/g) in eggs of Forster's tern (*Sterna forsteri*) from Lake Michigan were associated with a significant reduction in hatching success while no effect on hatching success was found at relatively lower median concentrations of 2,3,7,8-TCDD (8 pg/g) and total TEQ (201 pg TEQ/g) in eggs from the reference colony in 1983 (TEQs based on concentrations of 2,3,7,8-TCDD and dioxin-like PCBs only; Kubiak *et al.* 1989). Concentrations of PCB-126 in common terns embryos from the AOC were two orders of magnitude below the lethal dose associated with 50% embryonic mortality in common terns (104 ng/g based on egg injection; Hoffman *et al.* 1998). In addition, 2,3,7,8-TCDD concentrations were below those associated with decreased embryonic growth and edema in herons (150-250 pg/g; Hoffman *et al.* 1996).

Evidence suggests that herring gulls at the two AOC colonies and reference colony were exposed to PAHs based on detectable concentrations of parent and alkylated PAHs found in embryos of eggs incubated in the lab. It is difficult to speculate on spatial differences in exposure of gulls from the three

colonies since data are limited to a single pooled sample from each of the three colonies. Concentrations of the four parent PAHs detected in embryos were within the range of mean concentrations in eggs of predatory birds in Britain (Pereira *et al.* 2009). In a study of 24 PAHs injected into chicken eggs (Brunstrom *et al.* 1991), benz[a]-anthracene and benzo[k]-fluoranthene were among the most toxic compounds with LD_{50} values of 79 ng/g and 14 ng/g, respectively. Concentrations of these parent compounds in gull eggs were at least two orders of magnitude lower than these LD_{50} concentrations. Exposure to mixtures of PAHs however may result in additive toxicity and little is known with respect to their combined effects. Furthermore, given that PAHs and to a lesser degree alkylated PAHs are not bioaccumulative, it is difficult to relate body burdens with exposure. Although diet is generally assumed to be the main route of exposure, airborne exposure of particulate-bound PAHs may be an important route of exposure. It is noteworthy that higher concentrations of the alkylated PAHs relative to parent compounds were found in all samples. Alkylated PAHs are not typically quantified and little is known about their toxic effects in biota. These compounds may have greater toxicity than the parent compound as was demonstrated in one study for benzo[a]-anthracene (Marvanova *et al.* 2008).

Exposure to high concentrations of mercury can have significant impacts on reproductive success in birds and can also result in teratogenic effects in avian embryos, as demonstrated in egg injection studies with methylmercury in the laboratory (Fimreite 1974; Hill *et al.* 2008; Heinz *et al.* 2011). Overall, mercury concentrations in all embryos were below the predicted threshold of 0.6 µg/g (wet weight) in eggs as being protective against adverse reproductive effects for 95% of non-marine avian species (Shore *et al.* 2011). Two common tern embryos from North Sister Rock and the Cousins Island approached this concentration in 2012 (0.57 µg/g and 0.59 µg/g, respectively). Common terns however may be less sensitive to the effects of mercury exposure relative to other species. Based on the results of egg injection studies with methylmercury, both common terns and herring gulls exhibited medium sensitivity to mercury compared to 24 other avian species where embryo survival was examined (Heinz *et al.* 2009); other studies have also demonstrated species-specific differences in mercury sensitivity (e.g., Braune *et al.* 2012). Fimreite (1974) found no effect on hatching success in common terns at a colony where the mean mercury concentration in eggs (1 µg/g) was at least twice those found in this study. While no deformities were found in the two embryos from North Sister Rock and Cousins Island, sublethal effects that impair chick growth, behavior or survival cannot be ruled out however and these may be apparent in birds with relatively higher mercury burdens. Relative to mercury concentrations in herring gull eggs from other Great Lakes colonies in 2011 and 2012, mercury concentrations in gull eggs from the AOC colonies ranked 10th out of 15 herring gull colonies monitored (ranked as means from highest to lowest; EC unpublished). For both herring gulls and common terns, there was no evidence of significantly higher mercury concentrations in embryos from the St. Marys River AOC compared to reference colonies. These results suggest that current mercury concentrations would unlikely impact reproduction of breeding herring gulls and common terns in the St. Marys River AOC.

Elevated concentrations of PCBs and dioxins in eggs were associated with increased incidences of morphological deformities observed in wild populations of colonial waterbirds in the 1970s-1990s (Gilbertson *et al.* 1976; Ludwig *et al.* 1996). In 1998, Michigan State University researchers found three cross-billed common tern chicks out of 120 birds at the colony on Lime Island (EC *et al.* 2002). In this study, surveys of herring gull and common tern chicks at both AOC and reference colonies revealed no

evidence of morphological deformities in both study years, a finding which is consistent with low organochlorine and dioxin burdens found in embryos from these sites. In the artificial incubation study however, embryonic deformities were detected in a single embryo from one or more AOC colonies per year, but out of a small sample size of fertile embryos. The detection of a limited number of deformed embryos, but not free-living chicks, may not be surprising since affected individuals in wild populations would likely die before hatching or shortly thereafter and therefore not be captured in a deformity survey. It is somewhat puzzling however that deformed embryos were found only at AOC colonies and not at reference colonies for both species in both years. Specifically, three deformed herring gull embryos were found out of 56 fertile eggs (5%) and two deformed common tern embryos were found out of 56 fertile eggs (4%) when counts for AOC colonies and study years were combined. No deformed embryos were observed out of 46 fertile herring gull eggs and 25 fertile common tern eggs examined at the reference colonies, Double Island and Cousins Island, in the two years, respectively. Similar concentrations of non-*ortho* PCBs, 2,3,7,8-TCDD, and TEQs between deformed and normal embryos from AOC colonies of both herring gulls and common terns rule out the possibility that embryonic deformities were associated with exposure to dioxin-like PCBs and dioxins. Given the low probability of detecting deformed embryos at colonies, two additional years of egg collection and artificial incubation were conducted in 2013 and 2014 to increase sample size and examine this further. The results are outlined in the Addendum of this report and summarize four years of embryonic deformity data for both species. Based on these findings, deformities were evident in embryos from both AOC and reference colonies for both species and at comparable frequencies between the two groups. There is no evidence to suggest that there were differences in developmental effects in herring gulls and common terns that could be associated with influences that are specific to the St. Marys River AOC.

Reproduction based on productivity values for these two species in the St. Marys River AOC was considered good for herring gulls and was within a range considered typical for common terns in this region. Productivity of herring gulls at all study colonies was consistently high and above 1.3 chicks per nest in both study years. This was in the upper range of productivity levels required to maintain a stable herring gull population (range=0.8-1.4 chicks per nest; Kadlec and Drury 1968). Overall productivity of common terns at both the AOC colony and reference colony was low with comparable estimates of 0.40 and 0.45 chicks produced per nest, respectively, in the two study years. These rates are well below those required to maintain a population (1.1 chicks per nest; DiCostanzo 1980). However, these rates are consistent with low productivity observed as part of an intensive study of site tenacity and productivity in common terns conducted by EC throughout the North Channel in 2010-2012 (Moore and Weseloh 2012). In that study, the overall mean annual productivity of common terns in the region was 0.34 chicks per nest with a range in rates at six colonies in three study years from 0 - 1.0 chicks produced per nest. Productivity estimates for North Sister Rock and Cousins Island cited in this report are included in this overall mean annual productivity value. Low productivity in the North Channel was attributed primarily to egg loss and nestling loss caused by severe weather events (e.g. flooding) and predation by birds and mammals. The latter was evident at Hay Point in 2011 where significant predation led to abandonment of this as a nesting site. These results suggest that low productivity observed for common terns in 2011 and 2012 were not localized to the St. Marys River AOC but rather were consistent with low productivity observed in terns in the North Channel during this period. In an earlier study in the St.

Marys River, Michigan in 1980, mean productivity of common terns at 12 colonies was also low at 0.36 chicks fledged per nest (Scharf 1981). Habitat loss associated with high water levels and flooding, erosion of natural and made-made islands, and encroachment by vegetation at nesting sites in the St. Marys River were cited as factors contributing to reduced reproductive success at those nesting sites. Overall, external stressors appear to be largely limiting productivity of terns in the AOC and the region. As effectively demonstrated in the artificial incubation study, intrinsic factors (e.g., contaminants) did not impact hatchability of eggs as embryonic viability was high for both terns and gulls at study colonies in the two years.

Stable isotopes of nitrogen and carbon are used to provide information on trophic position and carbon source in the food web, respectively (Hobson 1999). For both herring gulls and common terns, the results of these analyses indicate that there were distinct and consistent differences in diet between birds from AOC colonies and those from reference colonies in the two study years. Significantly higher $\delta^{15}N$ values in embryos from the reference colonies in 2011 and 2012 suggest that the breeding birds from the reference colonies occupied a higher trophic level compared to birds from AOC colonies. Specifically, gulls and terns at the reference colonies may have fed more on fish (or larger fish) compared to birds from AOC colonies which fed at a relatively lower trophic level. For gulls, this could include terrestrial food sources such as small mammals, refuse and plant material (Fox *et al.* 1990). This apparent difference in trophic level between the colonies may have contributed to higher concentrations of some compounds in terns from the reference colony compared to an AOC colony in 2011 and 2012. Based on the isotopic signatures for $\delta^{13}C$, gulls from reference colonies may have fed more on aquatic-based prey types (with more depleted $\delta^{13}C$ signatures) compared to gulls from AOC colonies which fed more on terrestrial-based prey types (with more enriched $\delta^{13}C$ signatures). This is consistent with the spatial pattern evident for $\delta^{15}N$ in gulls. In the case of piscivorous common terns, birds from the reference colony may have fed more on fish from offshore areas compared to terns from AOC colonies which fed on fish from more inshore areas. Determinations of total clutch volume and intraclutch variation in 3-egg clutches for both species were informative for evaluating potential food stress during the egg production period in the two study years. Similarities for these endpoints between AOC and reference colonies suggest that food availability was likely not limited for laying females.

Two additional endpoints relating to growth and development of chicks were also measured in this study. Corticosterone deposited in growing feathers provides important insight into the physiology of stress during the period of feather growth (Bortolotti *et al.* 2009). As an indicator of stress over time, comparable corticosterone concentrations were found in feathers of herring gull chicks between AOC colonies and the reference colony in both years. However, common tern chicks from the AOC colony had a significantly higher mean concentration of corticosterone in feathers compared to reference colony chicks in 2011. Possible stressors could include low food availability, inclement weather, and/or threats of predation that may have been more prevalent at the AOC colony. Food availability may not have been an issue for terns in the AOC based on preliminary analyses of body condition of tern chicks and further supported by the clutch data for breeding females during the egg-laying period. Thyroxine concentrations in plasma of herring gull chicks were comparable among study sites in 2011 suggesting that there were no differences in chick growth and development or possible endocrine disruption among colonies.

In conclusion, the results of this two-year study suggest that there is no evidence of impaired reproduction or deformities in colonial waterbirds attributable to local contamination effects within the St. Marys River AOC (Ontario). Embryonic viability of herring gulls and common terns was high at AOC colonies. Herring gull productivity at AOC colonies was sufficiently high to maintain a stable population. While considered low for common terns, productivity at AOC colonies was consistent with that in the region and was largely attributable to external stressors such as predation and severe weather events. No morphological deformities were found in $\geq$ 21-day-old chicks of either species. Frequencies of embryonic deformities were comparable between AOC colonies and reference colonies for both species based on additional collections of eggs artificially incubated in the lab (as summarized in the Addendum). Importantly, contaminant burdens (e.g., PCBs, 2,3,7,8-TCDD, and mercury) in gull and tern embryos from the St. Marys River AOC (Ontario) were not notably elevated and were comparable to burdens at respective reference colonies in 2011 and 2012. Concentrations of PCBs, other organochlorine compounds, PBDEs, dioxins and furans, and mercury were not sufficiently elevated to adversely impact the reproductive success and development of herring gulls and common terns foraging in the St. Marys River AOC.

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APPENDIX 1. Mean concentrations (SD) of 4 non-*ortho* PCBs, 6 mono-*ortho* PCBs, and 17 furans and dioxins in embryos of herring gulls (HERG) and common terns (COTE) collected from the St. Marys River (SMR) AOC and reference colonies (i.e., Double Island and Cousins Island) in 2011 and 2012 and incubated in the lab (pg/g, wet weight). Concentrations are shown for deformed embryos (analyzed as individuals) and normal embryos based on the analysis of pools consisting of five individuals. Accordingly, N represents either the number of deformed individuals or the number of pools analyzed.

Compound	HERG-SMR AOC		HERG-Double I	COTE-SMR AOC		COTE-Cousins I
	Deformed N=3	Normal N=3	Normal N=1	Deformed N=2	Normal N=2	Normal N=1
PCB-77	249.07 (384.48)	61.90 (71.18)	36.70	617.25 (135.41)	548.50 (9.19)	1000.00
PCB-81	120.10 (53.96)	72.30 (9.52)	98.90	55.58 (25.49)	48.05 (5.73)	75.80
PCB-126	699.00 (149.79)	497.67(70.12)	680.00	389.75 (259.15)	346.00 (82.02)	455.00
PCB-169	112.70 (54.67)	92.07 (20.57)	121.00	68.03 (37.16)	70.20 (18.95)	85.20
PCB-105	48102 (5660)	42113 (8771)	59149	18067 (8249)	17970 (279)	16877
PCB-114	3273 (245)	2754 (116)	3150	1045 (502)	974 (131)	713
PCB-118	109721 (12333)	94632 (5426)	116622	45638 (28654)	47239 (10551)	34511
PCB-156	15096 (1125)	15201 (285)	19236	7543 (6161)	7803 (3437)	4891
PCB-157	2609 (129)	3149 (299)	3732	1931 (1512)	1906 (1029)	1251
PCB-189	2468 (945)	2724 (389)	3299	1531 (663)	1716 (261)	1478
2378-TCDD	4.76 (0.51)	3.30 (1.30)	5.62	2.49 (1.84)	2.14 (0.64)	3.80
12378-PeCDD	4.15 (1.93)	3.13 (0.94)	4.49	4.49 (3.24)	4.37 (1.75)	5.87
123478-HxCDD	0.39 (0.25)	0.27 (0.22)	NDR	1.09 (0.53)	0.46 (0.64)	0.95
123678-HxCDD	5.78 (1.40)	4.85 (0.72)	5.26	3.38 (2.10)	3.99 (0.43)	3.68
123789-HxCDD	1.28 (0.50)	0.82 (0.16)	1.15	1.22 (0.45)	1.10 (0.34)	1.00
1234678-HpCDD	3.42 (0.86)	7.64 (3.44)	5.63	2.56 (0.33)	1.85 (0.66)	2.59
12346789-OCDD	8.07 (1.62)	16.26 (8.28)	11.6	5.03 (3.37)	2.33 (0.72)	2.57
2378-TCDF	0.55 (0.95)	^	^	6.55 (2.79)	4.51 (6.37)	18.10
12378-PeCDF	0.46 (0.80)	0.03 (0.04)	^	0.17 (0.23)	0.67 (0.94)	^
23478-PeCDF	2.92 (2.96)	2.44 (2.14)	3.17	5.11 (3.62)	4.54 (1.34)	4.95
123478-HxCDF	1.07 (0.43)	0.99 (0.46)	1.05	0.97 (0.61)	0.83 (0.32)	1.01
123678-HxCDF	1.43 (0.20)	0.96 (0.30)	1.64	1.17 (0.89)	1.07 (0.21)	1.11
234678-HxCDF	0.82 (0.57)	0.66 (0.19)	0.97	0.90 (0.37)	0.87 (0.06)	1.04
123789-HxCDF	NDR	0.09 (0.02)	0.21	0.17 (0.09)	0.11 (0.06)	0.12
1234678-HpCDF	NDR	NDR	5.05	0.13 (0.18)	0.04 (0.06)	0.12
1234789-HpCDF	0.18 (0.08)	0.09 (0.08)	NDR	0.07 (0.03)	0.19 (0.10)	0.16
12346789-OCDF	1.13 (0.51)	1.04 (0.52)	1.16	0.58 (0.21)	0.52 (0.05)	0.53

NDR = Not detected due to incorrect isotope ratio; ^ Possible chlorinated diphenyl interference

ADDENDUM TO Assessment of the Wildlife Reproduction and Deformities Beneficial Use Impairment in the St. Marys River Area of Concern (Ontario) Environment Canada (February 2014)

PURPOSE

This addendum provides the results of further assessments of embryonic deformities in artificially-incubated herring gull and common tern eggs collected from St. Marys River AOC (Ontario) colonies and reference colonies in two additional study years, 2013 and 2014. As outlined in this assessment report, deformed embryos were observed at AOC colonies in artificial incubation studies conducted in 2011 and 2012 while no deformed embryos were found at reference colonies in both study years for either species. Given that the purpose of this study was to discern whether adverse effects in wildlife could be associated with influences within the St. Marys River AOC, this difference in frequency of embryonic deformities relative to reference colonies was of interest and further investigations were conducted in 2013 and 2014 as recommended by Environment Canada researchers. The probability of detecting an embryonic deformity was low overall at colonies and an increased sample size of incubated eggs would elucidate this finding and demonstrate whether these frequencies are in fact comparable between AOC and reference colonies.

METHODS

Herring gull eggs were collected from the two St. Marys River AOC colonies, Hay Point and Pumpkin Point, and the one downstream Lake Huron reference colony at Double Island in 2014. Common tern eggs were collected from one new AOC colony, Hurt Rock (46^{o}19'42"N, 83^{o}55'23"W), in 2014 and from several downstream Lake Huron reference tern colonies in the North Channel in 2013 and 2014. These included the one tern colony where eggs had been collected previously in 2011 and 2012 at Cousins Island (2013, 2014) as well as three additional colonies at Henry Island (45^{o}54’14"N, 82^{o}47'04"W; 2013 and 2014), Robb Rocks (46^{o}08'30"N, 82^{o}43'25"W; 2013 and 2014), and Tug Reef (46^{o}08'29"N, 82^{o}44'03"W; 2013). Figure 1 shows the location of all gull and tern colonies where eggs were collected for artificial incubation over the four year study period. The total number of eggs collected from each colony in 2013 and 2014 ranged from 24-30 eggs for gulls and from 1-32 eggs for terns.

As described in the methods of this report, unincubated eggs were collected from gull and tern colonies, transported to NWRC and then artificially incubated in the laboratory until just prior to the pipping stage of development at which time they were opened and embryos were examined. All embryos were scored for the presence of physical deformities and counted.

RESULTS

In 2014, no embryonic deformities were found in incubated herring gull eggs from the two AOC colonies at Hay Point and Pumpkin Point (44 fertile eggs in total) while three deformed embryos were found out of 17 fertile eggs (17%) at the Double Island reference colony. Deformities in these three individuals included one embryo with an upper mandible recessed in the skull, one embryo with a shortened lower mandible and one embryo with a missing left eye, crossbill and shortened upper mandible. In three

Figure 1. Colony locations where eggs were collected for herring gull and common tern artificial incubation studies from 2011-2014. St. Marys River AOC (Ontario) colonies include Hay Point and Pumpkin Point for herring gulls and Hay Point, North Sister Rock and Hurt Rock for common terns. Lake Huron reference colonies in the North Channel include Double Island for herring gulls and Cousins Island, Henry Island, Tug Reef (circle obscured) and Robb Rocks for common terns.

study years, this is the first year in which a deformed gull embryo was found at the Double Island reference colony.

There was no evidence of embryonic deformities in incubated common tern eggs from the AOC colony at Hurt Rock in 2014 (of 14 fertile eggs in total). Four deformed embryos were found out of 51 fertile eggs (8%) collected from two of four reference tern colonies in 2013 and 2014. One deformed embryo from Henry Island in 2013 had an incompletely formed skull and no eye and three deformed embryos from Cousins Island in 2014 each had a sunken eye. This was the first year in four study years at Cousins Island where a deformed tern embryo was found following artificial incubation of eggs.

A summary of the incidence of embryonic deformities in artificially-incubated eggs of herring gulls and common terns collected from St. Marys River AOC colonies and Lake Huron reference colonies from 2011-2014 is provided in Table 1. Overall, the incidence of embryonic deformities was remarkably similar at 3% for both herring gulls and common terns from AOC colonies over the three study years. Similarly, the incidence of embryonic deformities was comparable at 5% at the reference colonies for both species over the study period.

Table 1. Summary of the incidence of embryonic deformities in artificially-incubated eggs of herring gulls and common terns collected from St. Marys River AOC colonies and corresponding Lake Huron reference colonies in the North Channel from 2011-2014. Colonies are grouped according to their location (i.e. within the AOC boundary or reference colonies outside of the AOC) and collection years are indicated.

Species	AOC/Ref	Egg Collection Years	No. Fertile Eggs	No. Embryos Deformed	Embryos Deformed (%)
Herring Gull	AOC [a]	2011, 2012 & 2014	100	3	3%
Herring Gull	Ref [b]	2011, 2012 & 2014	63	3	5%
Common Terns	AOC [c]	2011, 2012 & 2014	70	2	3%
Common Terns	Ref [d]	2011-2014	76	4	5%

[a] Hay Point and Pumpkin Point; [b] Double Island
[c] Hay Point, North Sister Island, and Hurt Rock [d] Cousins Island, Henry Island, Robb Rocks and Tug Reef

CONCLUSIONS

Over a four year study period, embryonic deformities were evident at comparable frequencies at St. Marys River AOC colonies and reference colonies and equal to 3% and 5%, respectively, for both species. The additional two years of study, with increased sample sizes of incubated eggs, resulted in increased statistical power for estimates of frequencies of embryonic deformities in these two species under controlled laboratory conditions. The results of this study indicate that frequencies of deformities cannot be linked to either the geographical area where eggs were collected or to contaminant burdens as demonstrated in the comparisons of dioxin-like PCB, furan and dioxin burdens between deformed and non-deformed embryos from AOC colonies in this report. Based on four years of study, there is no evidence to suggest that there were differences in developmental effects in herring gulls and common terns that could be associated with influences that are specific to the St. Marys River AOC.